PANCREATIC INSUFFICIENCY IN CHILDREN WITH CYSTIC FIBROSIS

- A PROSPECTIVE OBSERVATIONAL STUDY

THESIS

SUBMITTED IN THE PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF M.D.PEDIATRICS

OF

DR. M.G.R MEDICAL UNIVERSITY, TAMILNADU, INDIA.

EXAMINATION TO BE HELD IN APRIL 2015

CERTIFICATION

This is to certify that the dissertation titled **PANCREATIC INSUFFICIENCY IN CHILDREN WITH CYSTIC FIBROSIS - A PROSPECTIVE OBSERVATIONAL STUDY** is the bonafide original work done by Dr. Archana Mitra M during her academic term June 2013 to May 2015, in the Child Health Department at Christian Medical College, Vellore in partial fulfilment of the requirement for the Master in Child Health examination of the Tamil Nadu Dr. M.G.R Medical University, Chennai to be conducted in April 2015.

Dr. Anna Simon, MD,DCH.

Professor and Head,

Department of Paediatrics,

Christian Medical College,

Vellore –632004

CERTIFICATION

This is to certify that the dissertation titled **PANCREATIC INSUFFICIENCY IN CHILDREN WITH CYSTIC FIBROSIS - A PROSPECTIVE OBSERVATIONAL STUDY** is the bonafide original work done by Dr. Archana Mitra M under my supervision during her academic term June 2013 to May 2015, in the Child Health Department at Christian Medical College, Vellore in partial fulfilment of the requirement for the Master in Child Health examination of the Tamil Nadu Dr. M.G.R Medical University, Chennai to be conducted in April 2015.

Dr. Sneha Varkki DCH,Dip.NB Paeds

Professor,

Department of Paediatrics Unit III

Christian Medical College,

Vellore – 632004.

CERTIFICATION

This is to certify that the dissertation **titled PANCREATIC INSUFFICIENCY IN CHILDREN WITH CYSTIC FIBROSIS - A PROSPECTIVE OBSERVATIONAL STUDY** is the bonafide original work done by Dr. Archana Mitra M during her academic term June 2013 to May 2015, in the Child Health Department at Christian Medical College, Vellore in partial fulfilment of the requirement for the Master in Child Health examination of the Tamil Nadu Dr. M.G.R Medical University, Chennai to be conducted in April 2015.

Dr. Alfred Job Daniel M.S Ortho.

Principal,

Christian Medical College,

Vellore –632004

DECLARATION

I , Dr.Archana Mitra. M, hereby declare that the dissertation entitled **PANCREATIC INSUFFICIENCY IN CHILDREN WITH CYSTIC FIBROSIS - A PROSPECTIVE OBSERVATIONAL STUDY** is the original work done by me during the academic term June 2013 to May 2015, in the Child Health Department at Christian Medical College, Vellore in partial fulfilment of the requirement for the Master in Child Health examination of the Tamil Nadu Dr. M.G.R Medical University, Chennai to be conducted in April 2015.

Dr.Archana Mitra.M

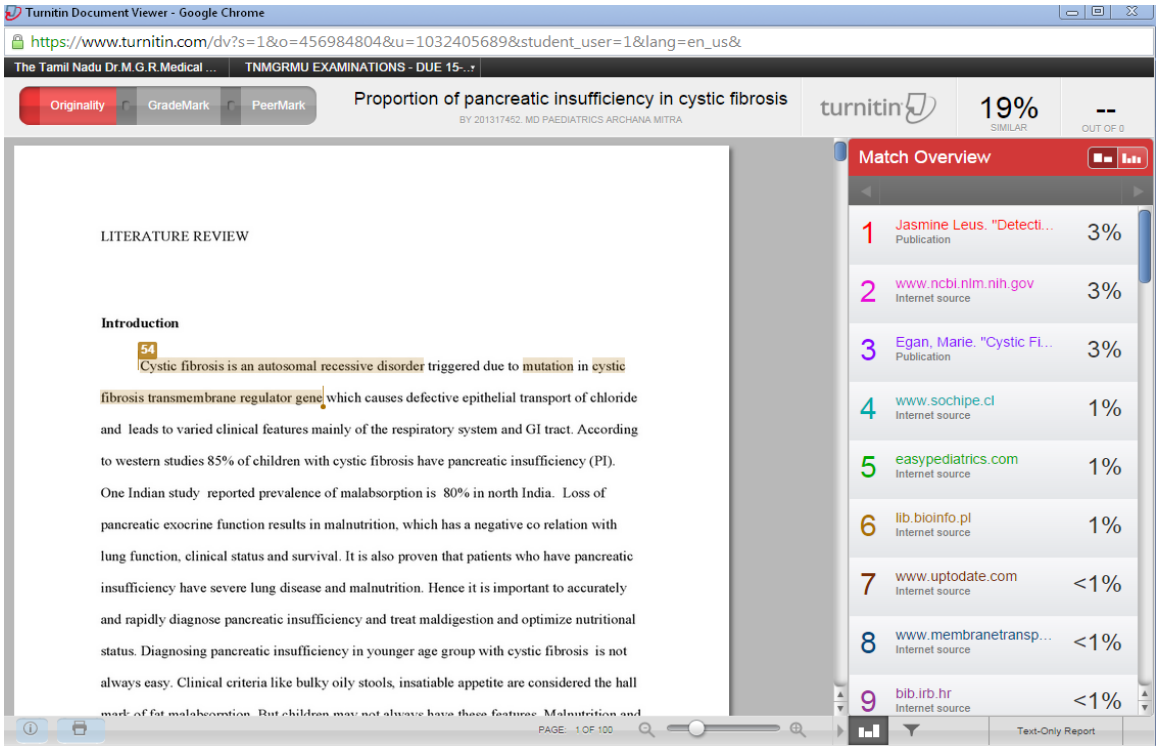
MD Paediatrics Final year (Post Diploma),

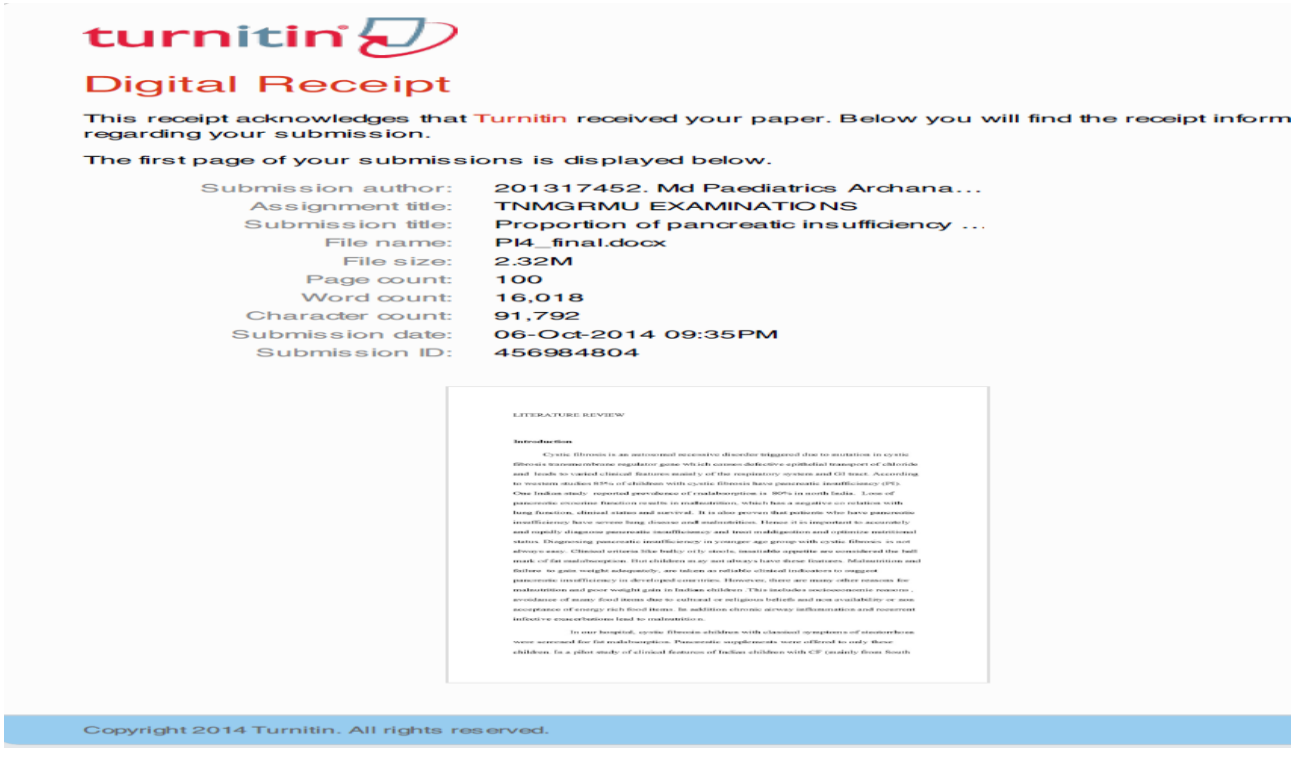
Child health department,

Christian Medical College,

Vellore 632004

ANTI- PLAGIARISM CERTIFICATE





ACKNOWLEDGEMENTS

This study could be carried out only due to the unswerving inputs and hard work of many individuals. I wish to place in record my sincere appreciation and immense gratitude to them.

To my guide**, Dr.Sneha Titus** for her constant support , patience and guidance throughout the study and helping me to perform this substantially.

Dr.Arul Premanand Lionel , my co guide for his support and guidance.

Dr.Jayasheelan for guiding me and also helping me in data analysis.

I am very thankful to our Biostatistician **Mrs. Thenmozhi** for her valuable inputs in statistical analysis.

CMC Vellore and all my teachers, for making this study and this course a reality.

I am grateful most importantly to all the patients without whom this study would not have been possible.

I warmly extend my gratitude to my husband Mr.Arun Kumar Kothapalli for his consistent help and support

My family, friends and colleagues for their love, constant support and encouragement.

Above all I thank, God for his abundant love and grace

CONTENTS

ABSTRACT

Introduction : In the caucasian population Cystic fibrosis is the most common life limiting genetic disorder with in incidence of approximately 1 in 2500 born in United Kingdom . 85% of those children have pancreatic insufficiency. There is paucity of data from India on the prevalence of cystic fibrosis,genetic profile of Indian patients and prevalence of pancreatic insufficiency.

OBJECTIVES: 1)To determine the proportion of children with pancreatic insufficiency among children with cystic fibrosis attending paediatric respiratory clinic in a tertiary care centre in south India during the period between September 2013-August 2014 .2)To compare the clinical and demographic characteristics of cystic fibrosis children who are pancreatic insufficient and pancreatic sufficient.3)To identify clinical variables towards development of a clinical score to predict pancreatic insufficiency.

METHODS : This prospective observational descriptive study was done on children with cystic fibrosis between the age groups of 0-15 years. After informed consent ,clinical and demographic data was collected using a structured proforma. Pancratic insufficiency was diagnosed if faecal elastase level was <200 microgram/ gram of stool . Prevalence of pancreatic insufficiency was calculated as percentage with 95% CI. Comparison between pancreatic insufficient and sufficient gruops were done comparing clinical variables using Fishers exact chi square test . Potential variables for score development are presented as RR (relative risk) variables after bivariate analysis which are included in multivariable regression analysis .Values with $p < 0.05$ are considered significant.

RESULTS AND CONCLUSIONS: Pancreatic insufficiency was present in 62.4% (95% CI 42.7-82.3) of 24 children . Additional 19% of patients were diagnosed to have pancreatic insufficiency by using faecal elastase test than what would have been detected if only classical history of steatoorhoea was used for diagnosis. Recurrent respiratory infections were present in 100% of patients while 78.8% had weight less than 5th centile on growth chart .Other classic features of cystic fibrosis like meconium ileus, rectal prolapse, nasal polyposis were seen in only a minority of patients. Presence of malodorous stool, frequent passage of stool and lower (worse)Cooperman score were more common amongst the pancreatic insufficient group compared to the pancreatic sufficient group and the difference was statistically significant. History of malodorous stool and frequent passage of stools (>/=3 times a day) were the two risk variables identified for computing a clinical score to predict pancreatic insufficieny. A larger study with a higher sample size will be required to develop this score.

Introduction

Cystic fibrosis (CF) is an autosomal recessive disorder caused by a mutation in cystic fibrosis transmembrane regulator gene which leads to defective epithelial transport of chloride and results in varied clinical features mainly of the respiratory system and GI tract. According to western studies 85% of children with cystic fibrosis have pancreatic insufficiency (PI). One Indian study reported that the prevalence of malabsorption is 80% in north India among cystic fibrosis children. Loss of pancreatic exocrine function results in malnutrition, which has a negative correlation with lung function, clinical status and survival. It is also proven that patients who have pancreatic insufficiency have severe lung disease and malnutrition. Hence it is important to accurately and rapidly diagnose pancreatic insufficiency and treat maldigestion and optimize nutritional status. Diagnosing pancreatic insufficiency in younger age group with cystic fibrosis is not always easy. Clinical criteria like bulky oily stools, insatiable appetite are considered the hall mark of fat malabsorption. But children may not always have these features. Malnutrition and failure to gain weight adequately, are taken as reliable clinical indicators to suggest pancreatic insufficiency in developed countries. However, there are many other reasons for malnutrition and poor weight gain in Indian children .This includes socioeconomic reasons, avoidance of many food items due to cultural or religious beliefs and non availability or non acceptance of energy rich food items. In addition chronic airway inflammation and recurrent infective exacerbations lead to malnutrition.

In our hospital, only those cystic fibrosis children with classical symptoms of steatorrhoea were screened for fat malabsorption. Pancreatic supplements were offered to only these children. In a pilot study of clinical features of Indian children with CF (mainly from South India) done in our instituition,only 26.7% were found to have symptoms of fat malabsorption(unpublished data).This is stark contrast to 85% prevalence of PI in Caucasian CF children. In a study consisting of children from Northern part of India and Pakistan prevalence of malabsorption was 80%(1) .The difference could be due to the difference in the causative mutation in the Indian population . Other possibility is that we are underdiagnosing PI , when diagnosis is based only on clinical features, Hence the need for this study to estimate the true proportion of PI amongst Indian CF children.

Stool elastase test , which is a sensitive and specific test for the purpose is costly and is not widely available. Many small hospitals will not be able to get this test done .Hence it is important to develop a scoring system based on clinical features and simple lab tests to reliably predict pancreatic insufficiency. This will be a useful tool for clinicians to identify those who present with PI and those who develop PI during the course of illness and start treatment early.

AIMS AND OBJECTIVES

AIMS AND OBJECTIVES

Primary Objective:

1. To find out the proportion of children with pancreatic insufficiency (PI) among Cystic Fibrosis patients aged 0-18 years attending a tertiary care centre in South India, using faecal elastase test.

Secondary Objectives:

- To correlate the demographic and clinical features with presence of pancreatic insufficiency
- To develop a clinical scoring system to predict pancreatic insufficiency in children with Cystic Fibrosis

LITERATURE REVIEW

Overview of Cystic fibrosis

Historical perspective

Cystic fibrosis (CF), is an autosomal recessive genetic disorder which has deleterious effects on vital organs like lungs, liver, pancreas and intestine. It is characterized by abnormal transport of sodium and chloride across an epithelium, leading to viscous and thick secretions. The name cystic fibrosis is given in reference to the characteristic scarring and cyst formation within the pancreas, and was first used in the 1930's. However the first description of cystic fibrosis dates back to 18th century when the literature from Germany and Switzerland warned the association between salt loss and the illness. Farber and Scwachmann postulated that cystic fibrosis is a generalised abnormality of mucus secreting glands and suggested the term mucoviscidosis(2).

It was in 1930 Dorothy Hansine Anderson published an article that first described cystic fibrosis . She described characteristic features of CF affecting pancreas. She also correlated the intestinal and lung findings which are characteristic of cystic fibrosis. She was the one to hypothesize that cystic fibrosis was a recessive disease and pioneered in the use of pancreatic enzyme replacement as a treatment for affected children.

In 1953 , 'Paul di Sant'Agnese' noted elevated salt content of sweat in people with cystic fibrosis after observing dehydration in children during a New York

City heat wave. He postulated that the concentration of sweat electrolytes were 2-4 times higher in cystic fibrosis and chronic pancreatic insufficiency. He also identified that the symptoms are initially due to pure salt loss and the subsequent loss of extracellular fluid volume(3).

The specific faulty gene which is responsible for CF was first identified in 1985 by Dr Francis Collins and Professor Jack Riordan(4). In early years nutritional support and airway clearance was the mainstay of treatment.

Subsequently 'Cystic Fibrosis Foundations Patient Registry' founded by Warren Warwick from 1964 had shown an increase in median survival from 14 years in 1968 to 20years in 1977(5). There was steady improvement in the condition and survival of many people with cystic fibrosis. New techniques of physiotherapy with new devices were described in 1980s when exercise was given more attention. The feasibility of eradication of Pseudomonas aeruginosa using nebulized tobramycin and colimycin was confirmed in a controlled trial done in Denmark(6), Later In 1983, Paul Quinton demonstrated that the chloride impermeability in sweat glands was the underlying basis for increased sweat electrolyte concentration in children suffering from cystic fibrosis(7)

In 1989 , the cystic fibrosis gene was detected by teams headed by 'LapCee Tsui' who termed the gene as Cystic fibrosis Transmembrane conductance regulator gene (CFTR) (8). Studies in early 1990's showed that Dornase alfa improves mucociliary clearance of CF sputum by breaking down extracellular DNA released from neutrophils which accumulates in response to chronic bacterial infection of airways(9). Subsequently clinical studies demonstrated the clinical safety and efficacy of dornase alfa in improving

lung function and decreasing pulmonary exacerbations.The first drug designed to target cystic fibrosis is the mucolytic Pulmozyne and the Food and Drug Administration approved the use of it(9). With early recognition of disease, aggressive treatment of malabsorption and respiratory infections, the mean life expectancy has improved from early childhood to 37.5 in 2005 .

Today, diagnostic tools from antenatal period is available. Antenatal screening, newborn screening and carrier testing of CF are being done. This screening tests along with good understanding of disease and treatment strategies, has given way to an extended life expectancy and better quality of life. Recent developments in medical research and technology will continue to provide hope for the future for care and drug therapy.

One such milestone is the development of Ivacaftor, a new drug that acts as a potentiator and activates defective CF transmembrane conductance regulator (CFTR) at the cell surface(10). The primary target for this therapy is mutated CFTR G551D.

Prevalence

World wide

Cystic fibrosis is the most commonly found genetic disorder in the white population accounting to 1 in 3000 live births(11). In the Asian Americans the prevalence is 1 in 31,000 individuals whereas in Asians it accounts to 1 per 90000. The estimated prevalence of CF among Indians residing in UK is 1 in 10,000 to 1 in 12,000 and in the USA is 1 in 40,000 respectively(12).

The prevalence of cystic fibrosis in South Asians was estimated to be 1 in 9200 as per CF database(13)

India

Cystic fibrosis was initially considered to be non prevalent in India as it was assumed that it was a disease of Caucasian population. Recent publications suggest that cystic fibrosis is more common amongst those of Indian origin than was previously assumed .Now it is clear that many cases are missed or underdiagnosed(1) . Indian children with cystic fibrosis are diagnosed in an advanced stage. It was in 1968 when the first case of cystic fibrosis was reported(14) . A recent study in India was done to account the prevalence of CF among Indians based on carrier frequency of del 508 mutations in samples of cord blood . The CF prevalence according to this study is 1/43,321 to 1/100,323.

Age Distribution

Age distribution of disease in any population is a function of birth rate , mortality rates and survical. According to 'Cystic Fibrosis Foundation, patient registry Yearly report 2004' in United States 42% were aged over 18, likewise 47% were aged above 18 in Canadian population.

Age at diagnosis:

In the west 70% of the CF patients are diagnosed before the first birth day and 90% before the eighteenth birth day(15). Late diagnosis there is usually associated with a milder clinical syndrome which includes a better lung function, nutritional status and a lower prevalence of colonization by Pseudomonas aeruginosa .

In Indian scenario the median age of diagnosis is 54 months (16) indicating the low index of suspicion for CF in India causing a delay in the diagnosis of the disease resulting in the high the morbidity and mortality. This caries implication when considering studies looking at long term benefit of neonatal screening for cystic fibrosis.

Pathogenesis

The fundamental pathophysiological mechanisms in cystic fibrosis include

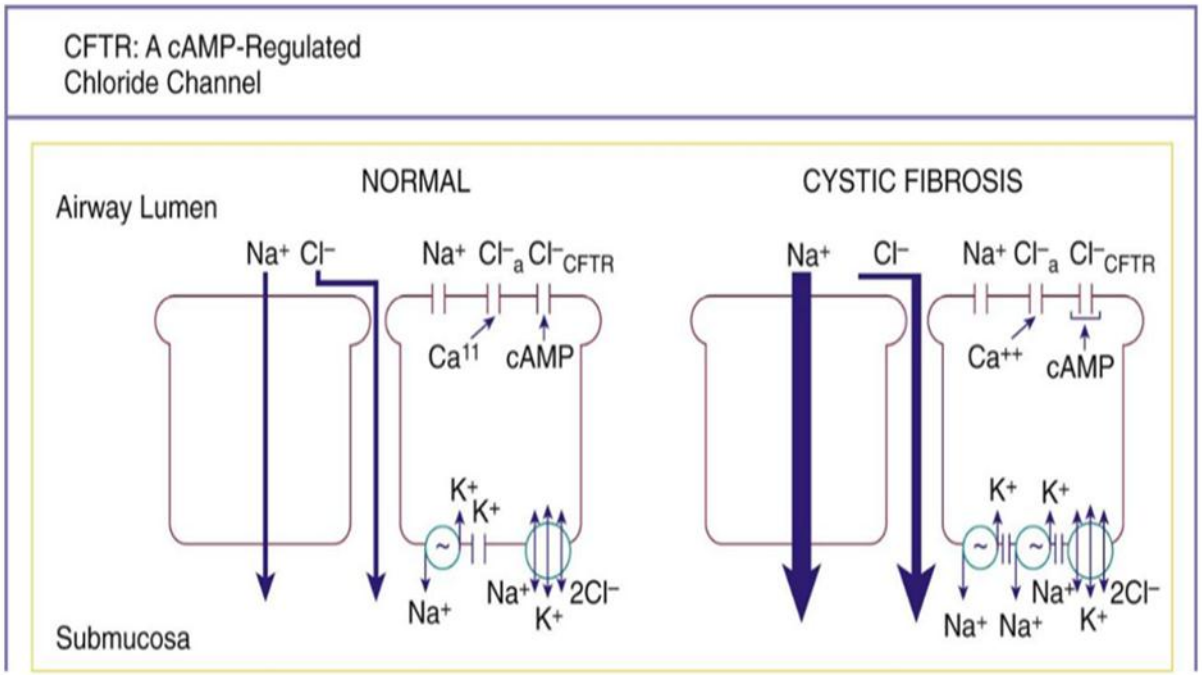
1) Failure to clear mucus secretions

2)Thick mucus secretions due to lack of water content

3) Raised content of salt in the sweat and other serous secretions

The basic underlying pathophysiological mechanism is that the epithelial cells membranes in CF are unable to produce chloride ions in response to cyclic adenosine monophosphate signals. The net result would be large quantity of sodium ions being absorbed through these epithelial membranes.

The following figure shows ‘ the net ion exchange across normal and cystic fibrosis airway epithelia under basal conditions’(17).



The expected net flux of water would be away from the lumen of the airway into the submucosa as water follows salt movement .This net flux would be more across CF epithelia. The raised sodium absorption by the CF cells is in association with a raise in amiloride sensitive sodium conductance across the apical membrane This causes raised Na-K ATP ase sites at the basolateral membrane .The c AMP mediated apical membrane conductance of chloride associated with CF transmembrane regulator (CFTR) has no role in CF epithelia, but an alternative Calcium activated chloride conductance is present in normal and as well as CF cells.It is also hypothesized that CF cells have reduced ability to produce chloride and absorb sodium in excessive amounts reducing the water present to hydrate secretions and allowing them to clear from airway lumen.

Classes of CFTR mutation

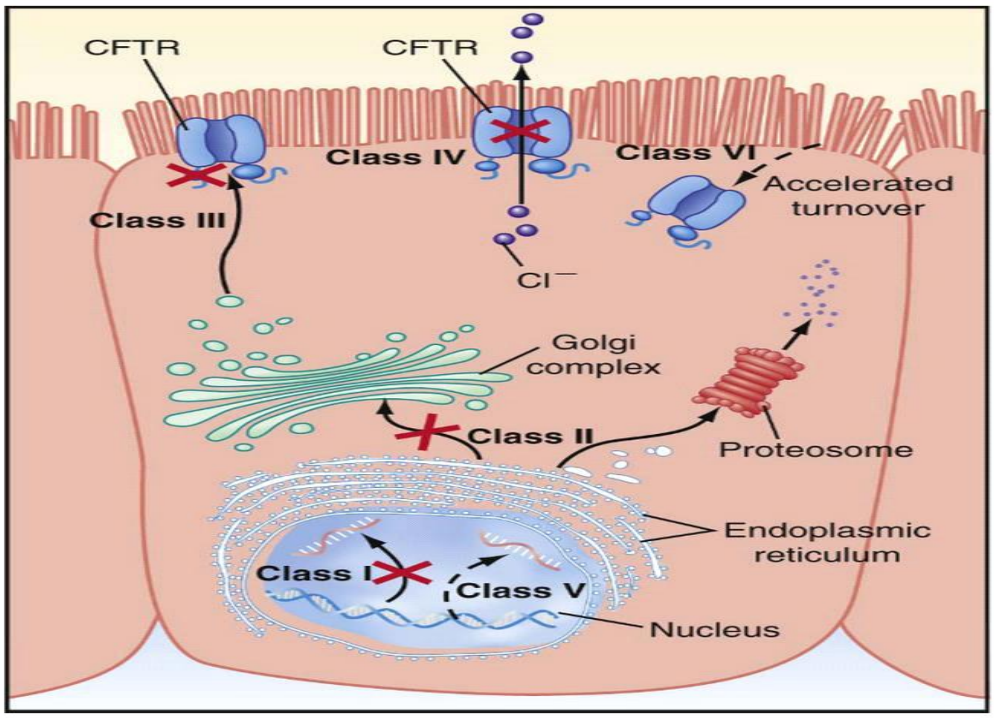
The primary function of CFTR is Cyclic AMP stimulated protein kinase A regulation of chloride conductance. In various mutations of CFTR this function is absent in the epithelial cells.

Table shows class of mutations, its effect on CFTR production and amount of CFTR available

Class of mutation	**CFTR production and function**
Class I	Deficiency of protein production with premature termination of CFTR protein generation. There is production of few functioning CFTR protein
Class II	CFTR Protein trafficking weakness so that it fails to reach the apical surface membrane where it is intended to function.
Class III	Defective regulation of gene which allows movement of chloride in and out of the cell. CFTR is not activated by ATP/cyclic AMP
Class V	Defect in splicing with decrease in production of normal CFTR

CFTR mutations fall into 6 classes along with some overlap . Those persons with class I, II, III on an average have decreased survival than class IV, and V which have mild genotypes.

The following figure shows categories of CFTRmutations(17)



MUTATIONS

In most European populations CFTR gene mutations are well characterized. In many Western- European countries, CFTR mutations were identified in greater than 95% of persons with CF.

The most common mutation causing CF is F508 del , the frequency of which is nearly 70% in many parts of Europe.

Few mutations, like 'F508del, N1303K, W1282X and 3120+1G>A' are predominant in Middle east countries and in many other parts of the world.(18).

F508del mutation is much more common in Europe as compared to the Middle East and it is relatively common in Israel and Lebanon countries as well. It is also noticed that, 3120+1G>A is more in individuals of African origin, which could have possibly spread from the African to Arabic populations.

Study done in AIIMS Delhi showed that the frequency of F508del mutation is 19% which is noted to be less than that was found in Caucasian population. 33.3% of F508del – homozygous mutation was shown in Mutation analysis study done in Kashmir (1) **.**

Classification of CF phenotypes(19)

1.Classical cystic fibrosis:

The clinical features of recurrent chest infections, malabsorption associated with pancreatic insufficiency, presentation in infancy with rectal prolapse, meconium ileus or unexplained malnutrition are included under classical CF

Pancreatic sufficient patients are not excluded from classical CF category although pancreatic insufficiency is seen in 85-90% of CF children. Positive sweat test and /or two CFTR mutations is diagnostic. Appropriate therapy for such patients should be ensured soon after diagnosis.

2.Atypical (non classic) cystic fibrosis

Children who do not present with full spectrum of clinical features associated with classical CF are grouped under atypical CF. There may be single organ involvement. Sweat testing may be normal or negative and CFTR analysis may reveal one, two or no

mutations. In patients with atypical CF who have two identified mutations one may be mild mutation resulting in partial CFTR expression and function.

3.CFTR related disorders

CFTR related disease is a term coined to classify ‘non CF conditions that carry higher incidence of CFTR mutations that could be expressed by chance but have no other indicators of either classical or atypical CF.

Examples include:

Allergic bronchopulmonary aspergillosis

Acute or recurrent pancreatitis

Isolated obstructive azospermia

Chronic rhinosinusitis

Disseminated bronchiectasis

Diffuse panbronchiolitis

Heat exhaustion

4.Genetic Predisposition for CF with no clinical sequelae

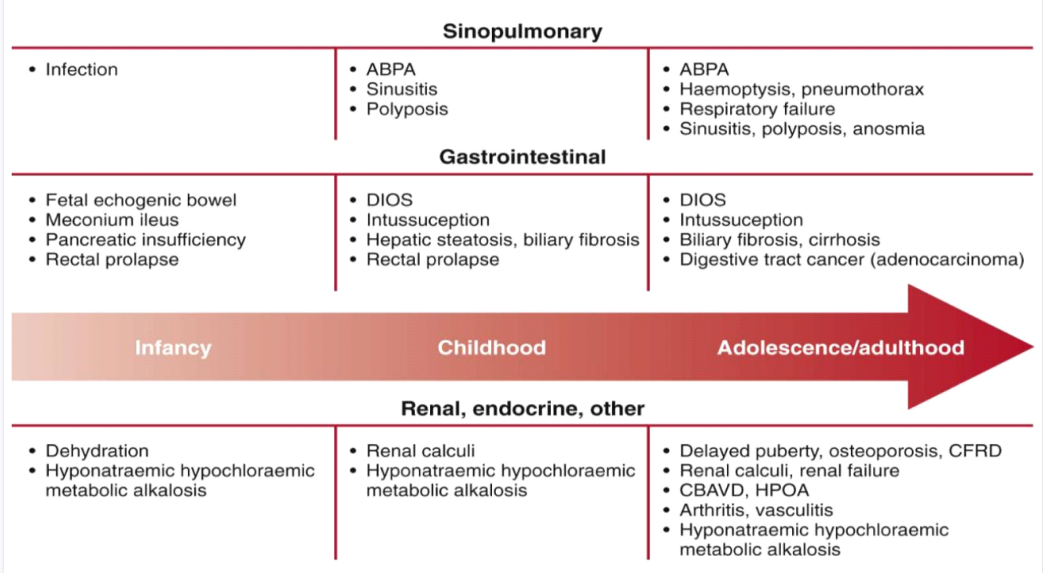
This referrers to group of individuals with the genetic potential to develop CF but in whom on detailed assessment there is no evidence of end organ dysfunction. There is a potential that clinical features will emerge with time but there is insufficient clinical

evidence to label the carrier of gene mutations with a disease. The role of prophylactic therapy in this situation is unclear.

Clinical presentation in cystic fibrosis

Symptoms related to cystic fibrosis may present at any time of life with tremendous variability and overlap of symptoms and timing between one patient to another.

The following figure shows average age of onset of clinical manifestations of cystic fibrosis in children(17)



Gastrointestinal symptoms

Meconium ileus is the neonatal manifestation of approximately 15% of cystic fibrosis infants. The frequency is more in siblings born following birth of a child having meconium ileus and is strikingly more common in monozygotic twins attributing to a genetic association from one or more genes(17) . Abdominal distension, vomiting, failure to pass meconium occur in first 24- 48 hours after birth. Abdominal radiographs

show distension of bowel with multiple air fluid levels and commonly presence of ground glass material in the lower abdomen. The presence of homozygous delta F508 deletion, in CF patients, is strongly associated with the presence of Meconium Ileus(20) and pancreatic insufficiency is found in all children homozygous for ΔF508. Gastrograffin enemas or surgery is needed to treat this condition. There have been no differences in survival between MI and non-MI patients as per recent articles.(21).

85-90% of children with meconium ileus develop pancreatic insufficiency which manifestes at birth or around the first year of life (16).

Biliary tract

Upto 30% of individuals with Cystic fibrosis have dysfunction of liver and is often recognised in the 1st 15 years of life . Symptoms of biliary cirrhosis are detected in only 5-7% of patients. Clinical manifestations of biliary tract include hematemesis from esophageal varices, icterus, ascites, and features of hypersplenism. There are reports of neonatal hepatitis picture as well(22). Cholelithiasis leading to biliary colic may be present in the 2nd decade or later. Liver disease is independent of genotype but is found in association with pancreatic insufficiency and meconium ileus. Cirrhosis, ascites, portal hypertension are the other hepatic manifestations of Cystic fibrosis.

Cystic fibrosis-related liver disease (CFLD) is defined if 'in a one year period, if at least 2 of the following findings are present on at least 2 consecutive examinations'

(1) "Ultrasound confirmed hepatomegaly;

(2) Elevated serum levels of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, gamma-glutamyltransferase; and

(3) Ultrasound abnormalities other than hepatomegaly like increased and heterogeneous echogenicity, nodularity, irregular margins, splenomegaly" icterus, ascites,.

Fatty liver on sonological examination is not considered as diagnostic criteria for CFLD.. If there are classic signs like splenomegaly, esophageal varices in addition to characteristic ultrasound findings of liver cirrhosis like coarse echotexture, nodularity, features of portal hypertension, evidence of porto systemic circulation, CFLD patients are classified as cirrhotics.

Pulmonary manifestations

Cough is the most constant symptom of pulmonary involvement which may be initially dry and hacking but subsequently becomes loose and productive. Expectorated mucus is usually purulent. Some patients remain asymptomatic for long periods or seem to have prolonged but intermittent acute respiratory infections. Others acquire a chronic cough in the first weeks of life, or they may have repeated episodes of pneumonia . Extensive bronchiolitis accompanied by wheezing is a frequent symptom during the first years of life. There are case reports on clinical presentation of CF as recurrent bronchiolitis in infants(23). As lung disease slowly progresses, exercise intolerance, shortness of breath, and failure to gain weight or grow become obvious . Exacerbations of lung symptoms very often require repeated hospitalizations for effective treatment. Cor pulmonale, respiratory failure, and death eventually supervene unless lung transplantation is

accomplished. Colonization with B. cepacia and other multidrug-resistant organisms may be associated with particularly rapid pulmonary deterioration and death.

The rate of progression of lung disease is the chief determinant of morbidity and mortality. The course of lung disease is largely independent of genotype . Severe mutations tend to be associated with more rapid progression. A few mutations may substantially or even fully spare the lungs. Male gender and exocrine pancreatic sufficiency are also associated with a slower rate of pulmonary function decline(17)

Early physical findings include increased antero posterior diameter of the chest, generalized hyper resonance, scattered or localized coarse crackles, and digital clubbing. Expiratory wheezes may be heard, especially in young children. Cyanosis is often a late sign. Common pulmonary complications include atelectasis, hemoptysis, pneumothorax, and cor pulmonale which usually appear beyond the 1st decade of life.

Respiratory infection and pathogens

The organism most frequently isolated from the sputum culture in cystc fibrosis patients is Pseudomonas aeruginosa. The prevalence of P.aeroginosa infection varies between countries and treatment centres within countries. Of all the factors that affect the infection rates the most important factor is the age .The prevalence of Pseudomonas, Burkholderia cepacia, Aspergillus species increase with age. Staphylococcus aureus colonisation remains constant throughout life(19).

In United States , 57% of Patients had Pseudomonas in their sputum culture or other respiratory cultures in 2004. In the UK paediatric centres in 2003, colonisation rates ranged from 3% to 47%(19).

In an Indian study done by Kabra et al(24) P. aeruginosa was found as an 82% culture positive patients .Its rate of infection was significantly higher as compared to the other organisms in CF patients.

Paranasal sinus involvement:

Even though the paranasal sinuses are virtually always opacified radiographically, acute sinusitis is infrequent. Nasal obstruction and rhinorrhea are common can be either caused by inflamed, swollen mucous membranes or, nasal polyposis. Nasal polyps manifest between 5 and 20 yr of age. The mean age of survival of people with cystic fibrosis worldwide has increased from 2 in 1950s to 37 years currently with roughly half of all CF individuals being of adult age. Survival depends upon both pulmonary and nutritional status.

Gastrointestinal Reflux Disease

Gastroesophageal reflux (GER) is found to be common in children and adults with CF. GER is also associated with reduced pulmonary function. In individuals with CF it is still unclear whether increased incidence of GER the primary phenomenon or it is secondary to the disease. The pathophysiology of increased GER in CF is still not known.

Findings of the Study done at Cottingham, UK showed that although weak acid GER is common in children with cystic fibrosis . GER is the primary phenomenon and it is not secondary to cough. Around one third of the children with CF have bile acid in saliva. Bile acid in saliva is an indication for increased risk for aspiration. They

recommended that the impact of salivary bile acid and potential risk aspiration on individuals with CF needs to be investigated further(25).

It was also found that the chest physiotherapy given for postural drainage in different positions may exacerbate GER in infants with cystic fibrosis . Study done by Ralf G Heine et al in Royal Children's Hospital, Australia did not find any correlation between the severity of the lung disease and the severity of GER. All infants who had significant GE reflux found to have normal chest x rays. The pathological reflux seems to be present even before lung disease is established(26).

CF Related Diabetes

After 10years of age, hyperglycemia occurs more frequently. It is found to a higher prevalence in females and in individuals with F508del mutation. Ketoacidosis is uncommon. Impaired insulin secretion and insulin resistance are the underlying pathology in CF with hyperglycaemia. When the child becomes 8-10year of age, an annual modified 2 hour oral glucose tolerance test is advised as part of routine screening.. HbA1C level should be checked once a year (17).

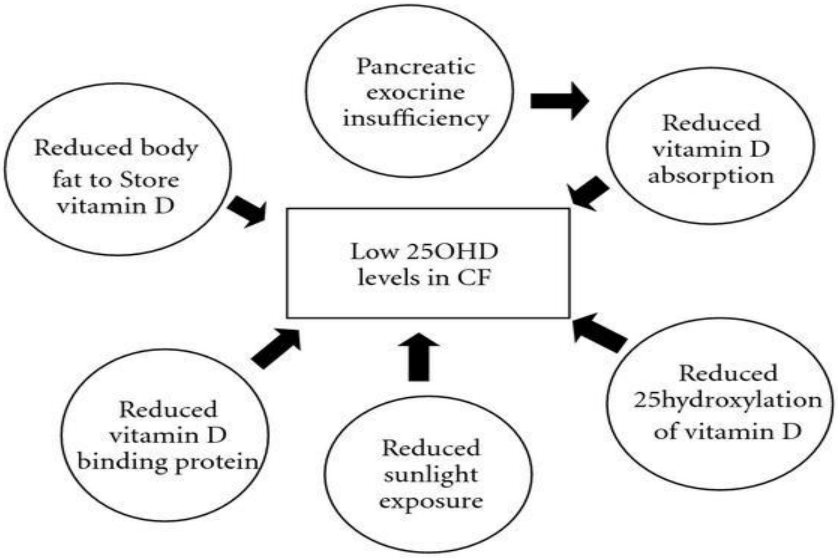
Vitamin D deficiency in cystic fibrosis

Vitamin D deficiency is common in individuals with cystic fibrosis due to impaired absorption of fat-soluble vitamins, decreased sunlight exposure, and suboptimal intake of vitamin D-containing foods and supplements. Vitamin D deficiency in cystic fibrosis has been associated with decrease bone mass in children and failure to achieve

adequate expected bone mass in adults. It has been shown to be the major contributor of bone disease in children(27). Also it may have impact on other comorbidities in cystic fibrosis. In a retrospective study done in Australia the prevalence of vitamin D deficiency in children with cystic fibrosis was found to be 15.54%(28).

Causes of hypovitamiosis D in cystic fibrosis:

The following figure demonstrates various causes of vitamin D deficiency in children with cystic fibrosis.



CF and Kidney Disease

CFTR gene is located in the proximal tubules of kidney, its dysfunction precipitates low molecular weight proteinuria. Kidney disease in CF is predominantly secondary to exposure to multiple aminoglycosides, immunosuppressants, non steroidal anti

inflammatory agents. Aberration in transport of sodium chloride, Pseudomonas colonization and development of Cystic Fibrosis Related Diabetes (CFRD) requiring insulin can result in nephropathy(3).

Evaluation of renal disease include measurement of serum creatinine and Creatinine Clearance,indirect measurement of GFR using Cystatin C,early indicators of Kidney disease like NGAL(Neutrophil Gelatinase associated lipocalin) and KIM(Kidney Injury Molecule)

Clinical Presentation of Cystic fibrosis in India

Indian Cystic fibrosis patients predominantly present with malnutrition secondary to respiratory and gastrointestinal problems. Amongst this varied clinical presentation, respiratory involvement is predominant manifestation(17). In one Indian study almost 94% of cystic fibrosis patients presented with respiratory symptoms followed by nutritional abnormalities and pancreatic dysfunction amounting to 21% and 4% respectively(24).In the same study the pancreatic abnormalities were observed primarily in older age group children CF children. Our unpublished data on clinical and genetic profile of CF children showed that among 22 CF children studied 86% had failure to thrive, 82% had recurrent respiratory tract infection, 41% had steatorrhoea at the time of presentation.

Diagnosis of Cystic Fibrosis

The diagnostic criteria for cystic fibrosis(29).

Presence of typical clinical features (respiratory, gastrointestinal, or genitourinary)

OR

A history of CF in a sibling

OR

A positive newborn screening test

PLUS

Laboratory evidence for CFTR dysfunction:

Two elevated sweat chloride concentrations obtained on separate days

OR

Identification of two CF mutations

OR

An abnormal nasal potential difference measurement"

Sweat chloride estimation for diagnosis of Cystic fibrosis

Children with CF secrete excessive salt in their sweat was reported by Dr. Paul A. diSant'Agnese in 1953 after observing dehydration in these children during a New York City heat wave (3)

Sweat test was introduced in 1959 and since then it has remained the "gold standard" diagnostic test for cystic fibrosis. Sweat testing quantifies the chloride level in the

sweat. Even though genetic mutation analysis are available, sweat chloride level estimation remains the standard test for diagnosing CF.

Pilocarpine iontophoresis is executed to collect the sweat and analysis of chloride content is done biochemically .

To carry out this test pilocarpine is injected into the skin of forearm to stimulate the sweat glands in the forearm.An electric current is used for the purpose. Sweat testing is difficult in the first two weeks of life in view of low sweat rates. But it is recommended that sweat testing can be done any time after the first 48hours of life (17)

Positive results should always be confirmed. If the test is negative, test should be repeated if suspicion of CF remains.

Interpretation of Sweat chloride results (30)

In infants more than 6 months of age, sweat chloride test result showing value less than or equal to 39 mEq/L probably suggests that cystic fibrosis is not present.

Value between 40 - 59 mEq/L implies that CF is likely to be present may not be conclusive and it suggests further testing is needed to confirm the diagnosis.

If the value is 60 mEq/L or more, it is diagnostic of CF when 1 or more other criteria is present

In individuals with normal exocrine pancreatic function the chloride concentrations in sweats are somewhat lower but usually it remain within the diagnostic range. In

conditions like eczema (atopic dermatitis), , malnutrition ,failure to thrive , ectodermal dysplasia ,deprivation are associated with false positive results whereas conditions like malnutrition, edema, insufficient sweat quantity, hyponatremia are associated with false negative results.

Diagnosis by Sweat Conductance

Sweat chloride estimation by pilocarpine iontophoresis requires great skill on the part of technician. Prevention of evaporation of sweat, determining sweat weight with chemical balance and calculation of chemical composition needs more precision and time. The macroduct sweat collection system avoids some of these problems. The macroduct sweat collection system consist of the Webster Sweat Inducer, Macroduct Sweat Collector and the Sweat –Check Analyzer where conductance is measured instead of chloride level.

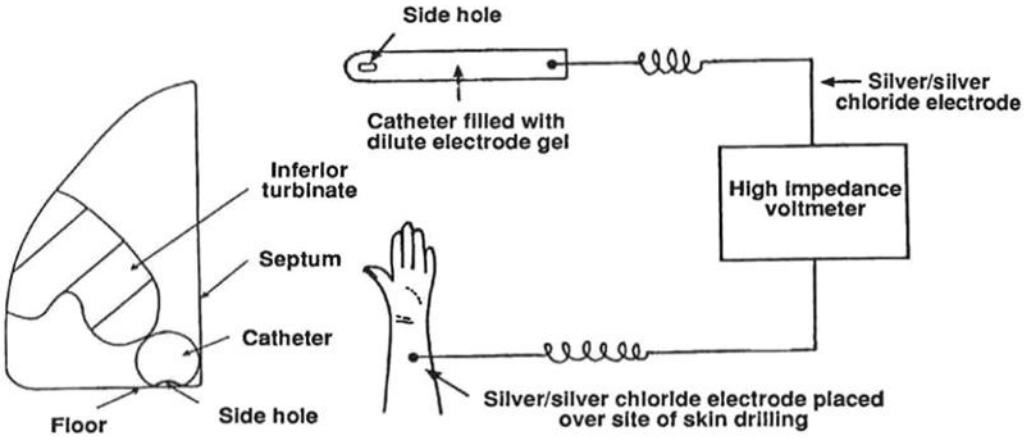
Readings are obtained in the following categories Upto 60 mmol/L – normal range, 60 to 80 mmol/L – borderline, >80mmol/L - abnormal . Applicability of this for screening for cystic fibrosis and as a diagnostic test has been verified in many studies(31). This commercially available test was used in our study for diagnosis of CF. In a study published in the Turkish journal of paediatrics, the results by these 2 methods were compared between 59 CF patients and 69 non CF patients. The Spearmen correlation test revealed strong correlation between conductivity test and chloride level ($r=88\%, p<0.001$)(32) .

Other method for sweat testing

Newer method of quantitative sweat electrolyte determination is CF Quantum test which is a swift and simple method .In a study done for dianostic accuracy of CF Quantum test the sensitivity of cystic fibrosis quantum test was 100% with 95% CI of 94-100% while the specificity was 96% with 95% CI of 89-99% respectively(33). However in three centre multicentre study the percentage of invalid tests was higher in the CFQT method (16.5%) compared to conventional sweat testing (3.8%) ($p<0.001$). CF Quantum test still requires refinement to improve the dianostic accuracy and reduce the number of valid tests.

Diagnosis by nasal potential difference:

The following diagram is the schematic representation of recording nasal potential difference and the anterior nasal cavity showing the site of measuring Nasal potential difference(34).



Nasal potential difference(NPD) is a test that quantifies the voltage difference across the nasal epithelium. It results from transepithelial ion transport and reflects in part CFTR function. This test certainly serves the purpose of diagnostic tool in cases where abnormal CFTR function is suspected and the other tests are inconclusive. NPD is research and diagnostic tool, and is used to assess the efficacy of new treatments such as gene therapy and ion transport modulators.

Genetic testing for CF

The CFTR gene is located on q31.2 locus chromosome 7. F508 del is the most common mutation caused due to deletion of 3 nucleotides resulting in loss of phenylalanine at 508th position on the protein..

Genetic testing for CFTR for diagnostic purposes is done when the sweat chloride concentration is unavailable or not confirmatory .

In the following distinctive conditions, Molecular testing of CFTR gene is regarded as the primary diagnostic test(35).

- Infants who have neonatal complications like meconium ileus as they cannot produce adequate quantity of sweat for sweat electrolyte test
- To test sibling of the affected patient who is symptomatic , to identify CFTR mutations in both.
- To test the high risk fetus in the antenatal period
- Antenatal diagnosis in low-risk fetus with ultrasound detected echogenic bowel

- Screening of the neonate

Targeted mutation analysis

5T/TG tract analysis

CFTR related disorders can be confederated with the poly T tract found on the exon 8 of the CFTR gene .5T, 9T and 7T are the three common variations of the poly T tract. 9T and 7T are considered as the polymorphic variations while 5T is aforethought as a variably penetrant mutation. The 5T variant is thought to decrease the efficiency of intron 8 splicing.

The TG tract lies just 5' of the poly T tract. It consists of a short string of TG repeats that commonly number 11, 12, or 13. A longer TG tract (12 or 13) in conjunction with a shorter poly T tract (5T) has the strongest adverse effect on proper intron 8 splicing'(36) .

Sequence analysis

Sequencing of all exons, intron/exon borders, promoter regions and specific intronic regions detects more than 98% of CFTR mutations(37).

Deletion analysis.

Multiplex ligation-dependent probe amplification can detect deletions not identified by aforementioned analysis. However, mutation detection rate is unclear.

NEWBORN SCREENING FOR CF

The rationale for newborn screening is that early detection of CF may lead to earlier intervention and improved outcomes because affected individuals are diagnosed, referred, and treated earlier in life as compared with individuals who are diagnosed after presenting with symptomatic CF.

Two methods are employed for new born screening in cystic fibrosis

Immunoreactive trypsinogen Assay:

Elevated levels of IRT are quantified by either Enzyme Linked Immunosorbent assay or radioimmunoassay. Levels of IRT fall rapidly during infancy, hence a negative test is not informative, the test being more sensitive than specific(38).

DNA assay:

Genetic analysis for mutations in the CF gene can be used as a primary or secondary screen to confirm and support the diagnosis in patients with non diagnostic IRT assays (IRT/DNA protocol)(39).

The purpose of a Neonatal screening (NS) for CF is to reduce the mortality and morbidity. The benefits of early diagnosis should countervail any possible disadvantage from screening of both the unaffected and affected babies. The NS programmes for CF are existing for more than 30 years now.. Clinical outcome studies by Doull et al have reported that there is decrease in hospital admissions during the first year of life and early improvement of nutrition for screened infants . Another systematic review

concluded neonatal screening of cystic fibrosis may results in better survival and outcome of the child (40) .

The neonatal screening along with identification of most asymptomatic patients offers the plausibility of early precautionary treatment and genetic counselling . The combination of IRT and PAP (pancreatitis associated protein) or Fecal elastase can be used for early screening of neonates.

Hurdles in Diagnosis of CF in India

Under diagnosis of Cystic fibrosis in India has various reasons. The respiratory manifestations mimic a variety of conditions like tuberculosis, bronchitis, pertusis, reactive airway disease , immune deficiency diseases , bronchiectasis, etc. The index of suspiscion is low in India due to high prevalance of infectious diseases like tubeculosis and reactive airway disease and the diagnosis of CF is missed . In addition to that the varied presentation and severity of disease further complicates the diagnosis of cystic fibrosis.Hence, CF may be far more common in people of Indian origin than what was previously thought but is under diagnosed or missed in majority of cases.

Sweat chloride estimation used as the diagnostic tool for suspected CF patients is not done in most parts of India due to lack of facilities .Two sweat chloride values are required as per diagnostic criteria which further makes it less suitable for Indian patients owing to the high cost of test. It is not cost effective to set up diagnostic facilities in many centers across the country. As the patient need to come to the facility for testing, it adds another hurdle in the diagnostic challenge.

The suspected CF cases with a borderline sweat chloride concentration (40-60 mmol/L) presents another diagnostic difficulty. It is not entirely clear if we can apply the same cut off values of sweat chloride to Indian population. It has been observed that many Indian patients with typical clinical features and sometimes positive CFTR mutation have lower sweat chloride level..Genetic mutation analysis can contribute to diagnosis of CF in case of intermediate sweat chloride levels(41).

MANAGEMENT OF CF

Cystic fibrosis is a multisystem disease which requires a holistic approach to care by multidisciplinary team. It is best done CF care centers involving physician, dietician, nurse, respiratory therapist, occupational therapist, psychologist. Nutritional and pancreatic insufficiency management will be discussed in detail later.

Pulmonary therapy

Antibiotics:

Course of pulmonary disease is heralded by chronic respiratory infections with periodic exacerbations. Oral azithromycin is recommended as prophylaxis. Long term treatment with nebulized antibiotics (Tobramycin and Aztreonam) particularly targeting against the common colonizing pathogens in CF like pseudomonas. This decreases the number of exacerbations, hospital admissions while improving improves the lung function..

Agents to promote airway clearance(19):

Inhaled DNA ase: cleaves the denatured DNA released by neutrophils that are degenerated. This thereby decreases the viscosity of pulmonary secretions.

Inhaled hypertonic saline

The high osmolality of the solution draws water from the airway epithelium to re-establish the aqueous surface layer which is deficient in CF.

Chest physiotherapy

It is strongly recommended to clear the airway secretions and is the mainstay of treatment of bronchoectasis.

vaccines

Pneumococcal and Influenza vaccine are recommended in all children with CF to prevent exacerbations

CFTR modulators:

Ivacaftor: It is recently designed drug to treat patients with a G551D mutation on at least one of CFTR genes. In phase 3 RCTs Ivacafter has improved the mean percent predicted forced expiratory volume in one second (FEV1), decreased sweat chloride levels and improved pulmonary symptoms resulting in weight gain.

Future Therapies for CF

Gene Therapy (19)

Cloning of CF gene can be performed though non viral and viral gene transfering agents and transferred to both bronchial and nasal epithelium.

Currently available non viral gene transfer agent is vector specific human CFTR. m RNA

Adeno Associated Virus is currently most commonly used vector for CF gene therapy , however repeated administration remains an unsolved problem

Scope of Stem cell therapy in relation to cystic fibrosis(19)

Following therapeutic measures are under research trials

- Stem cells derived from the bone marrow can be injected intravenously
- Topical administration of the exvivo corrected stem cells to lung
- Integrated viral vectors can be targeted on resident stem cells of the airway'

Pancreatic insufficiency in Cystic fibrosis

Pathophysiology of pancreatic insufficiency (PI)

Pancreatic exocrine insufficiency is major complication of CF and an important cause of maldigestion.. The normal digestive process involves stimulation and production of pancreatic juice by acinar cells and mixing of he undigested food with pancreatic juice with no duct outflow obstruction. Symptoms of malabsorption like oily stools, abdominal bloating, weight loss, would manifest if there is interruption in any of the steps

mentioned(42). Pancreatic enzyme secretion is stimulated during the cephalic and gastric phases to a certain degree, but the most important stimulation occurs during the intestinal phase, when chyme enters the duodenum.

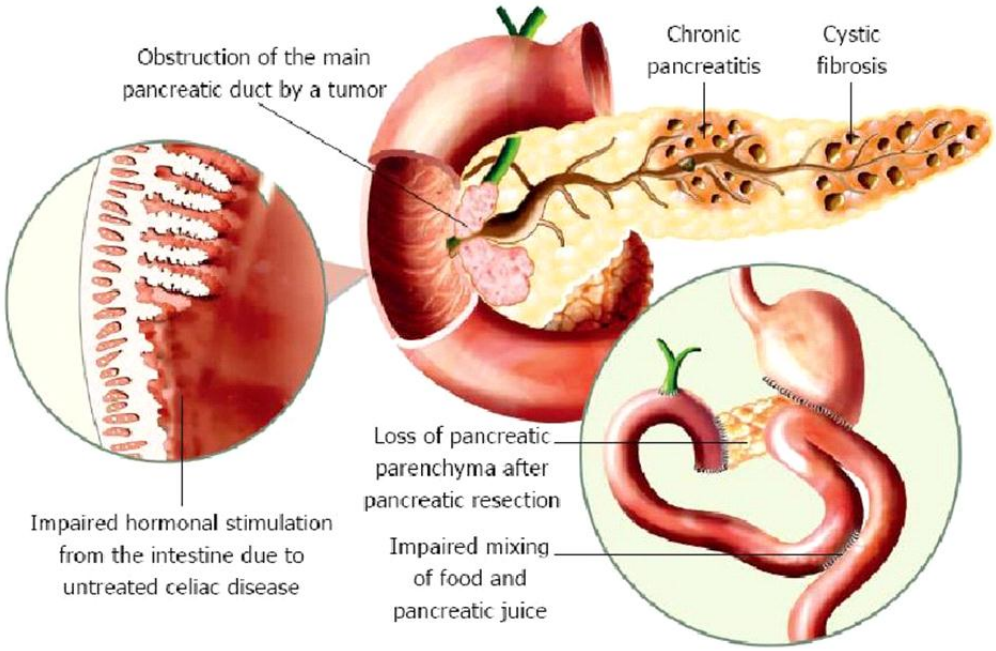
The presence of fatty acids, amino acids and gastric acid in the duodenum is the most potent stimulator of exocrine pancreatic secretion. Vagal and neural reflexes stimulate pancreatic secretion during the cephalic and gastric phases. During the intestinal phase, cells in the duodenal mucosa release CCK, which stimulates the secretion of pancreatic enzymes from acinar cells and secretin, which elicits water and bicarbonate secretion from ductal cells.

The pancreatic juice consists of bicarbonate and water secreted by ductal cells and several enzymes, secreted by acinar cells, with the specific capacity to digest proteins, carbohydrates and fat. In situations with reduced exocrine pancreatic function, the ability to digest fat is the determining factor that causes the most important symptoms and clinical complications because lipase, the major lipolytic enzyme of the pancreatic juice, is the pancreatic digestive enzyme with the poorest stability in the gastrointestinal lumen.

The destruction of lipase is even more rapid when the pH is below 4, which is often the situation in CF, in which the buffering of gastric acid is insufficient due to low bicarbonate excretion by the pancreas. Furthermore, there is minimal extra pancreatic lipolytic enzyme production, as opposed to the extra pancreatic capacity to digest carbohydrates provided by salivary amylase and intestinal oligosaccharidases or the proteolytic capacity provided by gastric pepsinogen.

Different causes of pancreatic insufficiency:

The following figure demonstrates various causes of pancreatic insufficiency (43)



Cystic fibrosis and pancreatic insufficiency

Patients with cystic fibrosis who are pancreatic insufficient have severe lung disease, malnutrition and liver disease. They are more prone to have pancreatitis as well .CFTR is an apical chloride channel located in the proximal duct epithelium that regulates active chloride transport across epithelial cell membranes and thus serves the function of pancreatic exocrine function. Deficient fluid secretion is apparent at all levels of pancreatic function and leads to pancreatic protein hyper secretion which may in turn result in protein precipitation and ductal plugging. An impaired chloride and bicarbonate secretion appears to account for this fluid secretion deficit. Individuals with pancreatic insufficiency have decreased or absent levels of pancreatic enzymes like amylase, lipase,

colipase and phospholipases. However, they have increased or normal production of salivary and brush border amylases, brush border peptidases and lingual lipases, which accounts for increased monosaccharide absorption, increased amino acid absorption, and few residual lipolysis respectively(44) .Due to the CFTR gene mutation , the chloride transport channel cease to function thereby resulting in accumulation of dehydrated thick protein-rich secretions which results in obstruction in the proximal ducts. This in turn results in secondary acinar cell destruction, fibrosis, and exocrine pancreatic insufficiency in 85% of the CF population .

60% of children with cystic fibrosis present with pancreatic insufficiency at birth. They present with symptoms of chronic diarrhoea, malabsorption, steatorrhoea, insatiable appetite ,poor weight gain and varying degrees of malnutrition(45). Pancreatic function in CF worsens with advancing age.

Genotype and pancreatic insufficiency

Pancreatic function has a direct correlation with the genotype of the CF patients. CFTR gene mutations may be classified as severe or mild with respect to functional status of the pancreas. Patients homozygous for two severe mutations experience severe clinical presentation including PI. Patients carrying at least one mild mutation such as R117H, 3171insC, A155P2, 138insL, 296 +IG-A, E92GK, E217G, 2789 +5G-A, or 3849 +10kbC-T and others are considered to be pancreatic sufficient (PS) and carry an overall prognosis that is vastly superior to CF patients with PI(46). Classes 1, 2, and 3 mutations responsible for little or no chloride channel function, confer the PI phenotype. In contrast, classes 4 and 5 mutations that allow CFTR residual

function confer the less severe PS phenotype. The PS phenotype occurs in patients who have one or two mild CFTR mutations, such as "R117H, R334W, R347P, A455E, and P574H", whereas the PI phenotype occurs in patients with two severe alleles, such as delta" F508, delta I507, Q493X, G542X, R553X, W1282X, 621 + 1G----T, 1717-1G----A, 556delA, 3659delC, I148T, G480C, V520F, G551D, and R560T"(47). Pancreatic insufficiency generally develops within the first few months of life in patients with two class I or II Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) mutations. These classes of mutation are characterized by defective production or processing of the CFTR protein, and include delta F508, N1303K, G542X, G551D and others .

Genotype and pancreatitis

Pancreatitis is an infrequent complication of cystic fibrosis. In one of the case reports published in 1975 pancreatitis was present in 0.5% of CF patients , all of them being pancreatic sufficient(48).In a large survey done among cystic fibrosis patients pancreatitis was diagnosed in 1.24% of patients of whom 56% were pancreatic sufficient and 25% had pancreatic insufficiency(49). This survey showed that patients with PS Cystic fibrosis had higher incidence of pancreatitis as a complication(10.3%), compared to patients with PI Cystic fibrosis (0.5%).

Sultan M et.al in their study on children with recurrent pancreatitis in Milwaukee, USA found that CFTR, SPINK1, or PRSS1 mutations were seen in 48%, 27%, and 24% of individuals with CF. 2 were homozygous for CFTR mutations, 6 were heterozygous and 4 of them had 5 T variants. 2 others patients had double heterozygous mutations in F508 del/2789 + 5G > A and F508 del/5T variant(50).

Indian studies showed that phenotype of Chronic Idiopathic pancreatitis is associated with SPINK1 and CFTR gene mutations in Indian population(51).

Diagnosis of Pancreatic Insufficiency in the context of Chronic pancreatitis (CP)

The classical clinical picture of PI is child presenting with foul-smelling, loose stools, weight loss, muscle wasting and flatulence. Advanced tests of pancreatic exocrine function can usually be avoided in patients with a well-established chronic pancreatitis diagnosis based on morphological findings and a clear clinical picture of pancreatic exocrine insufficiency (PEI). A trial of pancreatic enzyme replacement therapy (PERT) based only on the clinical picture is recommended by several national societies when the clinical presentation is strongly suggestive of PEI. However, only relying on symptoms may lead to both the over- and under-diagnosis of PEI. Diarrhoea and weight loss may be due to conditions other than PEI, and PEI can also be present in the absence of overt steatorrhoea.

In addition to explaining and treating clinical symptoms, the second rationale for the early diagnosis of PEI is to prevent complications of malnutrition. It is reasonable to assume that such malnutrition-related complications will be preceded by deficiencies of macro- or micronutrients detectable by routine blood tests. Theorotically serum nutritional markers can support the diagnosis of pancreatic insufficiency Deficiencies of several nutrients in blood tests have been demonstrated in CP, including apolipoproteins, total cholesterol, magnesium, lipid-soluble vitamins, retinol-binding protein, calcium, zinc and selenium, but the majority of these studies have not taken the exocrine function status of patients into consideration. Studies investigating the association between

nutritional markers and PEI in CP patients have demonstrated that deficiencies of lipid-soluble vitamins are associated with an increased probability of PEI(52,53), as opposed to B12 and folate levels, which are not associated with PEI(54).

The possibility of diagnosing PEI based on nutritional markers in the blood was recently studied in a cohort of 114 patients with Chronic pancreatitis, of whom 38 suffered from PEI (54).Hemoglobin, albumin, prealbumin and retinol-binding protein levels below the lower limit of normal magnesium levels below 2.05 mg/dL; and HbA1C levels above the upper limit of normal were all significantly associated with PEI. No PEI patient in this study presented with normal values for all of these parameters. The central conclusion that can be drawn from this study is that a normal panel of serum nutritional markers can exclude PEI with a high negative predictive value.

Radiological diagnosis for pancreatic insufficiency in relation to pancreatitis:

The probability of PEI in CP can also be estimated based on pancreatic imaging findings in the absence of more advanced tests of pancreatic function. Ductal changes on endoscopic retrograde pancreatography, computerized tomography and endoscopic ultrasound (EUS) have been associated with decreased exocrine pancreatic function.

The diagnosis of CP by EUS is based on the demonstration of several different parenchymal (hyperechoic foci, hyperechoic strands, parenchymal lobularity and cysts) and ductal (pancreatic duct dilatation, irregular pancreatic duct contour, hyperechoic pancreatic duct margin, dilated side branches and intraductal calcifications) abnormalities defined in the Rosemont classification (55). A recent study demonstrated a clear correlation between the number of EUS criteria met and the probability of PEI.

Calcifications and main pancreatic duct dilatation were independently associated with PEI in a multivariate analysis, and the probability of PEI was > 80% if these features were present(56).

Relation between nutrition and Lung function in CF

Patients with cystic fibrosis are not only known to have loss of fat in the stool but also have low energy intake in their diet . In the past all CF centres recommended low fat diet, until till mid 1980's when acid resistant pancreatic enzymes were available. Research done in Toronto had reported that calorie dense diet with no fat restriction given to patients with cystic fibrosis along with additional enzyme supplements, resulted in an improvement in the nutritional status and longevity among these patients .A similar study was done in Boston which is a comparably large and experienced centre but results obtained were inferior to the former study done in Toronto .

Longitudinal studies with sufficiently long follow up period have shown that young underweight patients have worst pulmonary function outcomes. Data from Cystic fibrosis foundation annual report shows that severe decline in FEV1(<40%) is associated with 61.2% of children whose weights are less tham 5th centile(57).

Thus in order to maintain pulmonary health and effective maintenance of its function aggressive nutritional support should be provided to all young cystic fibrosis patients with a goal to maintain normal growth patterns .

Improving nutrition and its effect on outcome of CF

An intervational randomized double blind placebo controlled trial done in Europe showed that twelve month nutrition intervention with organised lipid matrix has improved the growth status, muscle stores and Resting energy expenditure. Improvement

in nutritional status had a positive outcome in pancreatic insufficiency and mild lung disease(58).

Pancreatic insufficiency and it's relation to lung function in CF

CFTR gene responsible for the disease is located on the bronchial epithelium and gastrointestinal tract .In a cross sectional study (59) , Corey et al. in 1997 showed that the outcome among the cystic fibrosis patients with pancreatic insufficiency was directly related to the severity of the CF lung disease. This was further substantiated in study done by Schaedel et al where they concluded that many Pancreatic Sufficient patients had mild pulmonary disease in the long term, compared to patients with pancreatic Insufficiency(60). A cohort study done showed a significant difference(p=0.001) among patients with pancreatic sufficient and insufficient functional status in FEVI,FVC values(61). This individual group comparisions helped to demonstrated that patients with pancreatic sufficient status had consistently better lung function than compared to pancreatic insufficient status.

PI –relation to nutrition and growth pattern in CF

Pancreatic Insufficiency is the major contributor of nutritional problems in children with cystic fibrosis. The early growth pattern of infants with CF is dependent on both the age at diagnosis and the quality of the treatment they receive subsequently. Majority of the patients with pancreatic insufficiency manifest with early GI symptoms like oily stools, flatulence, bloating sensation etc., which if not recognized and treated early in infancy can lead to inadequate weight gain and complication secondary to malnutrition. In those

infants who were diagnosed by neonatal screening also tend to have have subnormal growth patterns during their first year if there was a delay in the start of treatment by more than a few weeks after birth Nutritional status in cystic fibrosis tends to decline during early child hood. Subsequent growth pattern after infancy is normal if the pulmonary infections are prevented and intestinal malabsorptions effectively treated(62).

Data from the Cystic fibrosis Foundation (CFF) shows that the body mass index (BMI) percentile typically begins to decline at about five years of age but does not cross the 50th percentile until about nine years of age(63).BMI target range for children with cystic fibrosis kis above 50^{th} centile. Children with BMIs between the 10th and 50th percentiles are generally considered at nutritional risk, and those with BMIs below the 10th percentile are in need of nutritional rehabilitation. Children with BMIs above the 85th percentile are considered overweight. Children who do no meet the targets for BMI and whose linear gowths are less than expected for growth potential should be given intensive counselling to optimize nutrition. Bone deformities account to one of the major co morbidities in children with pancreatic defieciency and poor nutritional status with high expected rates of fractures.

Tests of pancreatic function

Tests to detect the pancreatic secretion by direct stimulation with exogenous hormones

The pancreozymin-secretin test:

This test is based on the assumption that among the patients with cystic fibrosis pancreatic damage is the attributable risk factor that results in decrease in the capacity of the pancreas to secrete fluid, bicarbonate and enzymes.

In this test the duodenal juice is collected after an overnight fasting using a double lumen with the first opening at the ligament of Treitz and the second in the gastric antrum while continuous suction being applied to get uncontaminated duodenal juice.

Intravenous injection of secretin along with cholecystokinin is given to stimulate the pancreatic secretion , duodenal juice is collected for 10 minutes. The samples are examined for pH, bicarbonate and enzyme activities . This is the ``gold standard" test to quantify pancreatic function directly however the invasiveness and cost price of the test tend to discourage its routine clinical use(64).

Urine: indirect (tubeless) test of exocrine pancreatic function

The pancreolauryl test

Fluorescein dilaurate along with mannitol is given as an emulsion after an overnight fasting or after 4 hours of fasting for small babies .Panceatic aryl Elastase cleave fluorescein from fluorescein dilaurate ,free fluorescein is then absorbed in the gut , into the circulation and is finally is excreted in urine where it is measured spectrophotometrically. Mannitol resists hydrolysis in the stomach and small intestine and is excreted in the urine, where this can be measured enzymatically The flouresceine excreted in the urine is an indirect measure of pancreatic esterase activity while mannitol in urine is a measure of intestinal uptake. Results of this tests are measured in urinary fluorescein to mannitol ratios. These ratios are significantly lower in children with cystic fibrosis than healthy children (P=0.0001). The cut off of F:M rato etween pancreatic sufficient and insufficiency is 30 with the test sensitivity being 96% and specificity

95%.However this test cannot be used to identify some isolated enzyme deficiencies(65)

.

The N-benzoyl-1-tyrosyl-p-aminobenzoic acid test(66)**:**

N-benzoyl-l-tyrosyl- p-amino-benzoic acid (NBT-PABA), is a synthetic peptide which is selectively cleaved by chymotrypsin. Its excretion also depends on gastric emptying and absorption.Hence to quantify this 13c labelled PABA or PAS (Para amino salicylate) is used which follows the same path but not dependent of chymotrypsin). The test is done as follows:

Fasting urine sample before administration of 15mg/kg PABA and 4.5mg/kg PAS followed by 6 hr urine collection is performed.one. PABA and PAS concentrations are measured by gas chromatography-mass spectrometry(83). Results are given as an excretion index PABA/PAS. "The normal values are >0.6 (0.6±1.4) and CF patients have values <0.5". The results of the test can also be expressed as the percentage of the oral dose of NBT-PABA re-covered as PABA in the urine within 6±8 h. In healthy children the PABA recovery is more than 66%. Children with CF and exocrine insufficiency have results less than 15%.

Measurement of fat in the stool

Steatorrhea is classically defined as at least 7 g of fecal fat over 24 hours. Steatorrhoea can be due to other reasons than pancreatic insufficiency.

The titrimetric method:

The 72-hour quantitative fecal fat test is most abundantly performed test world wide to assess the pancreatic function. This test is based on saponification of faeces with

ethanolic alkali, liberation of fatty acids with hydrochloric acid, petroleum ether inturn extracts these fatty acids, separation of the petroleum ether and acid ethanol by the addition of sodium chloride and a small amount of amyl alcohol and estimation of the fatty acids in the petroleum ether fractions by titration(67). It indirectly estimates pancreatic function . It does not differentiate the different sites at which fat malabsorption is prone to occur such as the hepatobiliary, mucosal, and pancreatic causes for fat malabsorption. The disadvantage of this invstigation is that patients must stop pancreatic supplements during this study. The reason for this time period of 72 hours was in view of the varied stool fat excretion in a period of 24 hours , day to day which was demonstrated byVan de Kamer et al , thus 72 h stool sample is taken for accurate determination of stool fat. The important hindering factor experienced by the patients and laboratory technicians is the malodorous nature of the stools. Also, this 72 h collection becomes difficult in children when diarrhea is present due to the polyethylene lining present within the napkin(68)..

Steatocrit method:

In this method a stool sample is diluted with deionised water and homogenised and centrifuged. After cen-trifugation, the fat and solid layers are measured separately . Steatocrit is calculated as fatty layer.

This test was ameliorated by acidification of the stool homogenate with perchloric acid (5N for maximal acidification)(91) which results in a larger extraction of fat .

Microscopic examination.

A small amount of faeces is mixed with water and examined under high power microscopy (. An average of 2.5 fat droplets per high power field is seen in normal stool samples.. In CF, a large amount of round fat drops of different sizes can be seen.

The gold standard tests done for scrupulous assessment of the exocrine function of the pancreas is via direct measurements with the secretin–cerulein or secretin–pancreozymin test.The obstacles encountered with these direct functional tests are that they often carried out only at specialized centers, and they are time consuming and expensive.

Faecal immunoreactive lipase (IRL)

Stool samples must be stored at +/-20°C until analysis.. The lipase concentration is determined immunologically. Faecal IRL concentrations are age dependent. IRL in stool samples of patients with CF ranged from 0.03±107 lg/g (median 0.48 lg/g).

According to MuÈ nch et al., this test has a high sensitivity (87%) and an excellent specificity (97%) to detect pancreatic insufficiency. However, discrepancies in results obtained among different studies, which could be attributed to the different methods used to determine faecal lipase. The faecal IRL test is non-invasive and requires little co-operation by the patient or his parents in collecting the stool sample. The test can be repeated as many times required to support the diagnosis when the suspicion of exocrine pancreatic insufficiency is high. In comparision with most other tests done to support the diagnosis of pancreatic insufficiency such as the faecal chymotrypsin determination, 72 hour stool fat excretion the results of this test is not affected by pancreatic enzyme replacement therapy.

Fecal elastase for pancreatic insufficiency

Human pancreatic elastase is a glycoprotein synthesized by the acinar cells in te pancreas.It differs from other proteases of pancreas in that , besides extending proteolytic action , it mixes with bile salts and neutral steroids in the intestinal lumen thereby help in transport of cholesterol and its metabolites in the intestinal lumen.

This specific function of elastase is because of the stability during intestinal transit.Since elastase in faeces is five times higher than that in the pancreatic juice, it reflects the amount of pancreatic insufficiency accurately.(55). The results of this test is unaffected by exogenous pancreatic enzyme treatment.

In a study done to compare the efficacy of fecal elastase with chymotrypsin ,using a cut-off of 200 microg elastase-1/g, and fecal chymotrypsin (cut-off: 6 U/g) 'stool sensitivities of the same were 100% and 76%, respectively ($P < 0.0001$ and $P < 0.001$ respectively) in severe exocrine pancreatic insufficiency, 89% and 47% respectively ($P < 0.001$; $P = 0.34$, respectively) in moderate and 65% for both in mild pancreatic insufficiency. Specificities of elastase-1 and chymotrypsin in stool were 55% and 47%, respectively'. In the same study the positive predictive value of fecal elastase with cut off ofm 200µ/g of stool was provided as 50%.. Hence it was concluded that fecal elastase-1 is highly sensitive in the diagnosis of severe and moderate exocrine pancreatic insufficiency ,and is of significantly higher sensitivity than fecal chymotrypsin estimation .Because it is a measure of pancreatic function and not a measure of malabsorption, it is not valuable as a measure to monitor the effectiveness of Pancreatic enzyme replacement

therapy (55). Although, fecal elastase testing has high sensitivity and specificity in detecting severe pancreatic insufficiency in children with CF, this test performs less well for detecting mild or moderate pancreatic insufficiency, and also displays variability with repeat testing in this type of patient . Thus, results of fecal elastase testing should be combined with clinical observations, including nutritional status and symptoms of steatorrhea, to determine the need for PERT. Determination of fecal elastase can be performed on a single stool sample that requires no special storage, and does not require discontinuation of pancreatic enzymes. Thus, it is more clinically practical than a 72-hour collection of fecal fat or secretin stimulation tests. The results of fecal elastase testing correlate well with the secretin stimulation test and therefore is the gold standard for diagnosis of pancreatic insufficiency (69). The clinical evaluation EL-1 is stable in feces at room temperature during 1 week, at 4- 8°C during 1 month and at -22. 8°C for longer time.

Among the CF patients, in whom the clinical presentation is not very clear the EL-1 test may not be very reliable due to significant intra-patient variability. In one clinical study stool samples among the the CF subjects were collected for fecal elastase for 7 consecutive days where in the intraassay variability in CF patients was found to be 4.06%(70)

Fecal elastase as gold standard

Food and Drug administration has recently approved Fetal elastase1 a human monoclonal enzyme linked immunosorbent assay (ELISA) for diagnosing pancreatic insufficiency. Among the patients with cystic fibrosis, FE-1 has been proven to provide excellent results to support the diagnosis of PI, with a sensitivity of 98% to 100%and a

specificity of 93% to 100%, even while patients are taking pancreatic enzyme supplements(71). The ELISA is human elastase specific and therefore exogenous pancreatic enzyme supplements, which are of porcine origin, have no effect on the results .Human monoclonal elastase satisfies 5/6 criteria for an ideal pancreatic test. It does not get degraded during intestinal passage and correlates well with secretin pancreozymin test which is the most accurate test for determination of pancreatic function .FE1 is low in neonates and reaches normal adult values by age of 2 weeks(55).

Treatment of pancreatic insufficiency and outcome

The goal of treatment is to normalize digestion, alleviate PEI-related symptoms and prevent malnutrition-related morbidity and mortality and disease progression.

Historically, a low-fat diet has been recommended in PEI to reduce steatorrhea. This recommendation has been abandoned in modern dietary counseling in PEI due to the risk of aggravating PEI-related weight loss and deficiencies of lipid-soluble vitamins(72). By optimization of the PERT dose and supportive treatment with PPI it was observed that most PEI patients will tolerate a normal-fat diet. Hence, dietary consultation should include advice for sufficient caloric intake and normal fat content. Small, frequent meals are usually better tolerated than large, high-caloric meals. Deficiencies of fat-soluble vitamins are very common in PEI patients, and vitamin supplementation therapy should be given if necessary.

Pancreatic enzyme replacement therapy (PERT) in CF –history and current status

The Pancreatic Enzyme replacement therapy is advocated to be used in Pancreatic Insufficiency patients in order to maximize absorption of the nutrients especially lipids. In order to accomplish this goal this it is important to obtain adequate concentration of pancreatic enzymes in duodenum when the food is being delivered. In order to replicate this physiologic process resistance to gastric inactivation is necessary and delivery of active enzyme at the the duodenum, where digestion occurs is also a requirement.

Various formulations of PERT that were used in the past(73)**.**

1.**Pancreatin**, is a crude mixture form , which is derived from swine or ox pancreas.Each milligram contains 2 USP (United States Pharmacopeia) units of lipase and 25 USP units of amylase and protease activity.

2.**Pancrelipase** is the other formulation obtained from swine pancreas and is a more concentrated and purified enzyme preparation. Each milligram contains more than 24 USP units of lipase and 100 USP units of amylase and protease activity. Because of its higher enzyme content, pancrelipase formulations are favored over pancreatin preparations.

Currently, the main formulations are immediate-release, enteric-coated microspheres and minimicrospheres, enteric-coated microtablets, and enteric-coated microspheres with a bicarbonate buffer. Creon, an enteric-coated formulation of pancrelipase which is most studied and approved of all formulations of PERT delivered in the form of minimicrospheres.

Pancreatic Enzyme replacement therapy should be started if

1)the patient is known to have 2 CFTR mutations associated with PI

Or

2) the infant has unequivocal signs or symptoms of malabsorption, while awaiting confirmatory test results

Or

3) an objective test of pancreatic function indicates fat malabsorption(74).

Patients with supportive laboratory evidence of PI should be started on PERT even in the absence of signs or symptoms suggestive of fat malabsorption. Dosing of PERT is adjusted according to the amount of lipase in the supplements.The initial dose should aim at supplying 40 to 60 IU/minute of lipase activity within the duodenal lumen. For infants, PERT should be started at a dose of 2000 to 5000 lipase units for each feeding which approximately amounts to 120 mL. Dose of the PERT should be adjusted with increasing age to not more than 2500 lipase units per kilogram per feeding with a maximum daily dose of 10 000 lipase units per kg per day. Enzyme dose and rate of weight gain in relation to caloric intake should be evaluated and documented at each visit to monitor the efficacy of PERT.This is because the per kilogram dose of PERT and the volume of intake will rapidly increase in the first few months of life. PERT doses should not be increased beyond the upper limit of the recommended range because children are at risk of developing fibrosing colonopathy.

Monitoring the efficacy of PERT:

Currently there are no definite guidelines in clinical practice to monitor the efficacy of enzyme replacement therapy and to determine the need for dose adjustment. 72 hour

stool fat collection, coefficient of nitrogen absorption and various breath tests including 13C-labeled mixed triglyceride breath test have been described . Despite the tests for definitive diagnosis clinical decision is the common approach to start on enzyme replacement therapy and follow up of patients as stool collection is o time consuming , tedious and hard, and breath tests are not accessible to all patients due to its non-availability at all centres(73)

Adverse effects of PERT:

.Pancreatic enzyme replacement therapy is considered safe with limited adverse events when compared to placebo. The most commonly reported side effects for recently approved enzymes are headache (6%), dizziness (6%), abdominal pain (9%), and flatulence. Historically, hyperuricemia, and hyperuricosuria,dysuria and uric acid crystalluria have also been reported in cystic fibrosis patients with older formulations(74).

Scoring Systems used in CF

Total of 16 scoring systems have been described in literature for severity of cystic fibrosis(75). These are based on clinical evaluation, radiological and tomographic findings.These are grouped under Cysti fibrosis scoring systems(CFSS)

Of all these Schwachmann scoring system was a milestone in history of cystic fibrosis and is stil used as a classical tool for assessment of severity. This scoring system is based

on general activity, nutrition, physical examination and radiological findings.The lower the score, the severe is the disease(76).

NIH scoring developed in 1973 is critically analysed as simple,immediate and easily implemented(77). This included general condition, pulmonary and gastrointestinal manifestations(obstruction, poor absorption, abnormalities, sinusitis, nasal polyps) and complicaations including blood gas abnormalities.

First radiographic scoring was done by Norman and Chrispin based on characteristics on chest X ray (78).Following which many other scoring systems have been described which included clinical and radiological characteristics of CF.

Scoring based on HRCT findings of bronchiectasis was first developed by Nathanson. Higher scores corresponded to higher severity.

The most recently developed scoring system is by Kanga et al(79).It was critically analyzed to be simple, inexpensive and easily implemented. .It was designed to assess acute exacerbations of the disease, to predict improvement or worsening of pulmonary function of patients and to evaluate therapeutic effects.

A new surrogate measure for the seriousness of CFTR mutations, identified as Pancreatic Insufficiency Prevalence (PIP) score (80), was newly established and validated (Dorfman et al., 2010; Ooi et al., 2011a).This score is not to foresee pancreatic insufficiency, rather

to identify severity of CFTR mutation using the information of the prevalence of PI when individual mutation is existing in a patient. The PIP score for a specific mutation is the ratio between the PI patients resonant the mutation (Total PI), and all PI and PS patients (Total PI + PS) carrying the same mutation when in a Homozygous state or heterozygous amalgamation with F508del, G551D or class I mutations (bona fide severe mutations). For example,621+1G>T has a ratio of 1.00 which indicates that all patients with this mutationare PI. Likewise, a ratio of 0.1 R334W mutation means determines that 10% of subjects with this mutation are PI. .Nevertheless we did not come across any scoring system defined in the literature to foresee the pancreatic insufficiency built on clinical manifestations(81).

METHODOLOGY

METHODS AND METHODOLOGY

STUDY SETTING

Department of Paediatrics, Christian Medical College, Vellore

STUDY POPULATION

Children with diagnosis of cystic fibrosis aged 0-18 years as per inclusion criteria.

TYPE OF STUDY

Prospective observational descriptive study

STUDY PERIOD

October 2013 - August 2014

SAMPLE SIZE

Our primary objective was to study the proportion of pancreatic insufficiency among Cystic fibrosis children. Very few studies have looked at the prevalence of pancreatic insufficiency in India. Study done at AIIMS, stated that the prevalence of malabsorption was 80 %(108) which is consistent with the data from the west.

If we calculate the sample size using prevalence as 85% the number of CF patients required to study would be 51 based on the formula

4 pq/ d ^2,

Where ‘p’ is prevalence of pancreatic insufficiency ie 85%,

‘q’ is (100 – p) ie 15%,

d is margin of error ie 10

IRB and ethics committee approval

This study was approved by the Institutional Review Board (IRB), IRB Min No.8492, dated 9.10.2013 (Annexure 1)

INCLUSION CRITERIA

1.Children with diagnosis of cystic fibrosis based on elevated sweat electrolyte >80 mmol/lit (diagnostic range)by wescor macroduct sweat analyzer..

2.Those in whom diagnosis was based on clinical features and borderline sweat electrlyte level, (60-80 mmol/lit) , who either had typical features like bronchiectasis and pseudomonas colonisation or presence of one of common CFTR mutations on mutation analysis

EXCLUSION CRITERIA.

1. Those children who could not give stool sample for fecal elastase testing.

DETAILS OF METHODOLOGY

Study was done in Paediatric department in Christian Medical college Vellore from October 2013 till August 2014.Children diagnosed to have cystic fibrosis based on laboratory and clinical criteria from Paediatric Out patient department and wards who visited these areas during study period were enrolled .Patients in whom the diagnosis was newly made during period and those known cystic fibrosis patients who attended review clinic were enrolled.

No attempt was made to contact the old patients in the CF database as this may bias the results by preferentially recruiting pancreatic insufficient patients,.

Informed consent was obtained from the parent or guardian of the child and assent from the child where applicable. (Annexure 2 -5) Using a structured proforma(Annexure 6) information on clinical features and lab investigations was collected which included demographic data, clinical features pertaining to gastrointestinal as well as other systems, details of treatment. Height and weight percentiles were marked using Agarwal growth charts. Fresh stool sample was collected for faecal elastase examination .Stool samples were collected for microscopic examination for fat globules if they were not already on pancreatic enzyme replacement

Fecal elastase measurement – Methodology (Annexure 8)

Principle of the test:

The pancreatic Elastase ELISA is a solid phase enzyme linked immunosorbent assay (ELISA)based on a double sandwich technique applying two polyclonal antibodies

recognizing several different epitopes on defined species and organ specific human pancreatic elastase peptide sequences. The ELISA microplate is coated with antibodies directed against human pancreatic elastase binding the pancreatic elastase contained in different patient samples or in the standards, respectively.

In the next step the second antibody labelled with biotin binds to the immobilised pancreatic elastase.To visualise the bound pancreatic elastase, the biotin binds in the following step to streptavidin labelled horseradish peroxidase.The peroxidase then oxidises the substrate TMB (Tetramethyl benzidne).This reaction will be stopped by the addition of 0.25mmol/L of H_2SO_4.The developed dye (oxidised TMB) can be measured photometrically at 450nm.

Limitations of assay:

Watery stools from the patients may lead to falsely low readings because of the dilution effect

Estimation of faecal elastase 1 was done every 2 weeks in batches . The time taken for the test to be performed was 6 hours.

Expected values:

range	Fecal elastase in µg elastase /g of faeces
Severe exocrine pancreatic insufficiency	<100
Moderate exocrine pancreatic insufficiency	100-200
Normal exocrine pancreatic function	>200

.Another specimen of stool was subjected to microscopic examination for stool fat globules.

MUTATION ANALYSIS:

Genetic testing was done for few patients included in the study.

CFTR gene mutation screening for 4 common mutations namely F508del, G551D, G542X, 621G2T were done by Amplification refractory mutation system (ARMS) PCR .

.

DATA ENTRY AND ANALYSIS

Data entry was done by Epidata version 3.1 and analysis done by SPSS version 16.0.

The prevalence of pancreatic insufficiency and demographic groups are presented as number and percentage. Bivariate analysis is done for pancreatic insufficiency using chi square test with Yates correction. Risk estimates are presented as RR with 95% CI. The risk variables, are included in multivariable regression analysis. P value at 5% level considered as significant.

RESULTS

Following figure shows their state of origin.

Total number of patients enrolled are 24.

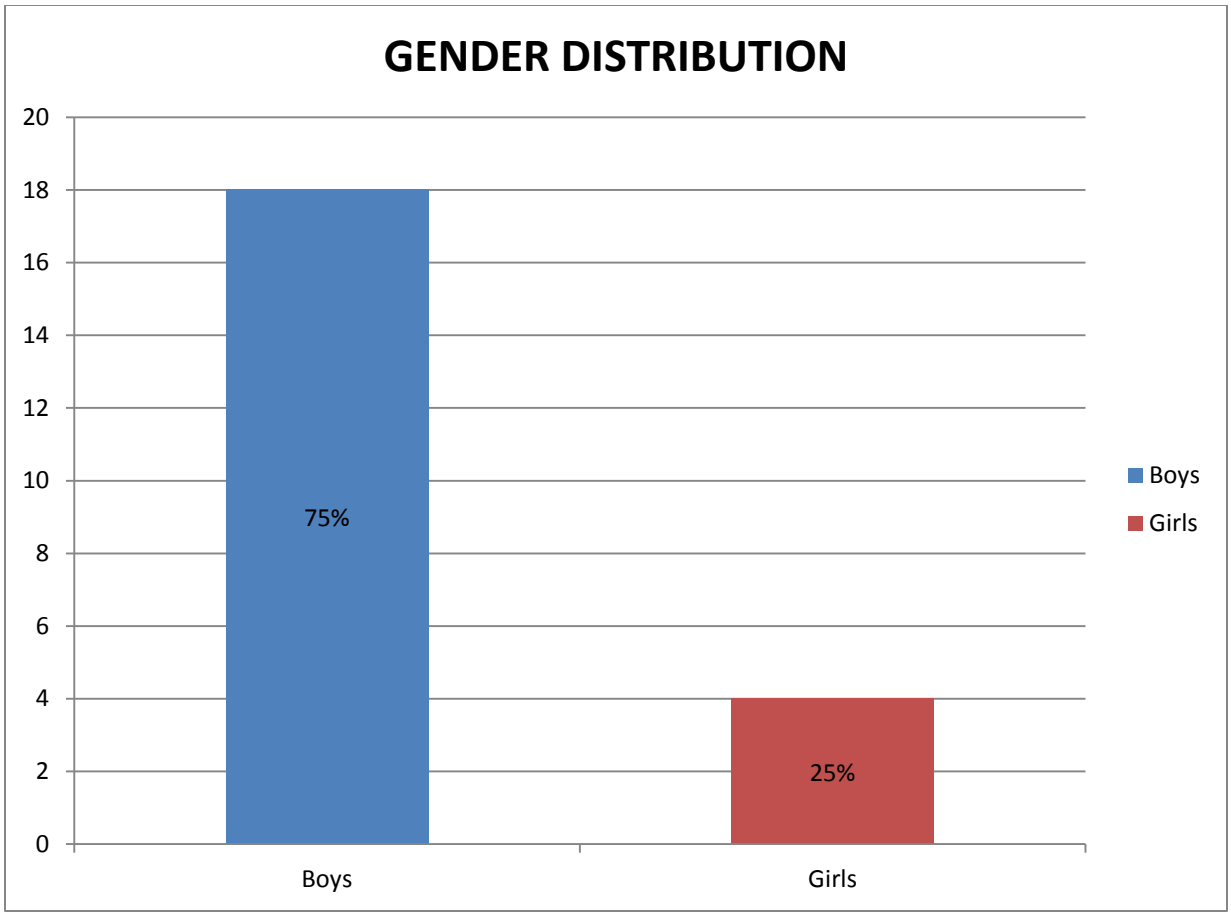


Fig.1. Gender distribution of subjects.

Out of 24 patients enrolled majority 18/24(75%) were boys and 25% were girls.

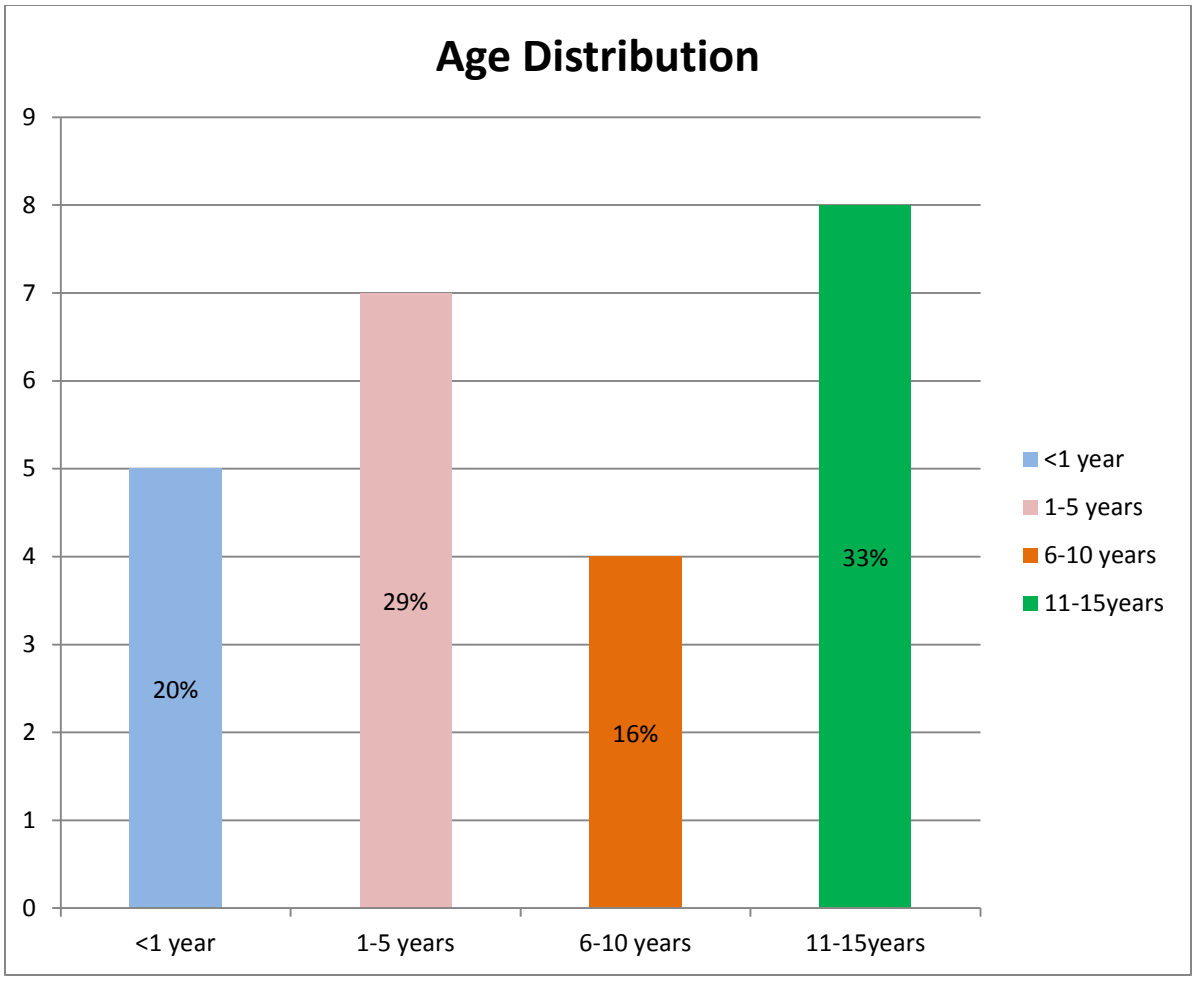


Fig.2 Age distribution of the children at enrollment .

The average age is 2.5 years +/-11 months

AGE AT THE ONSET OF SYMPTOMS

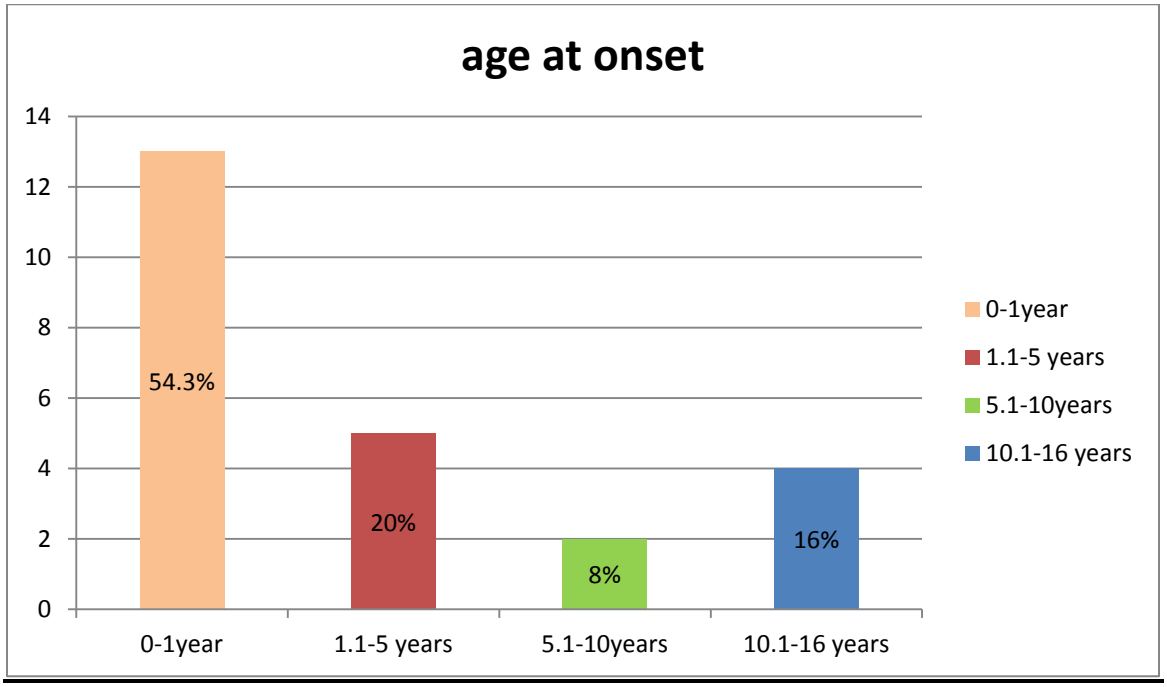
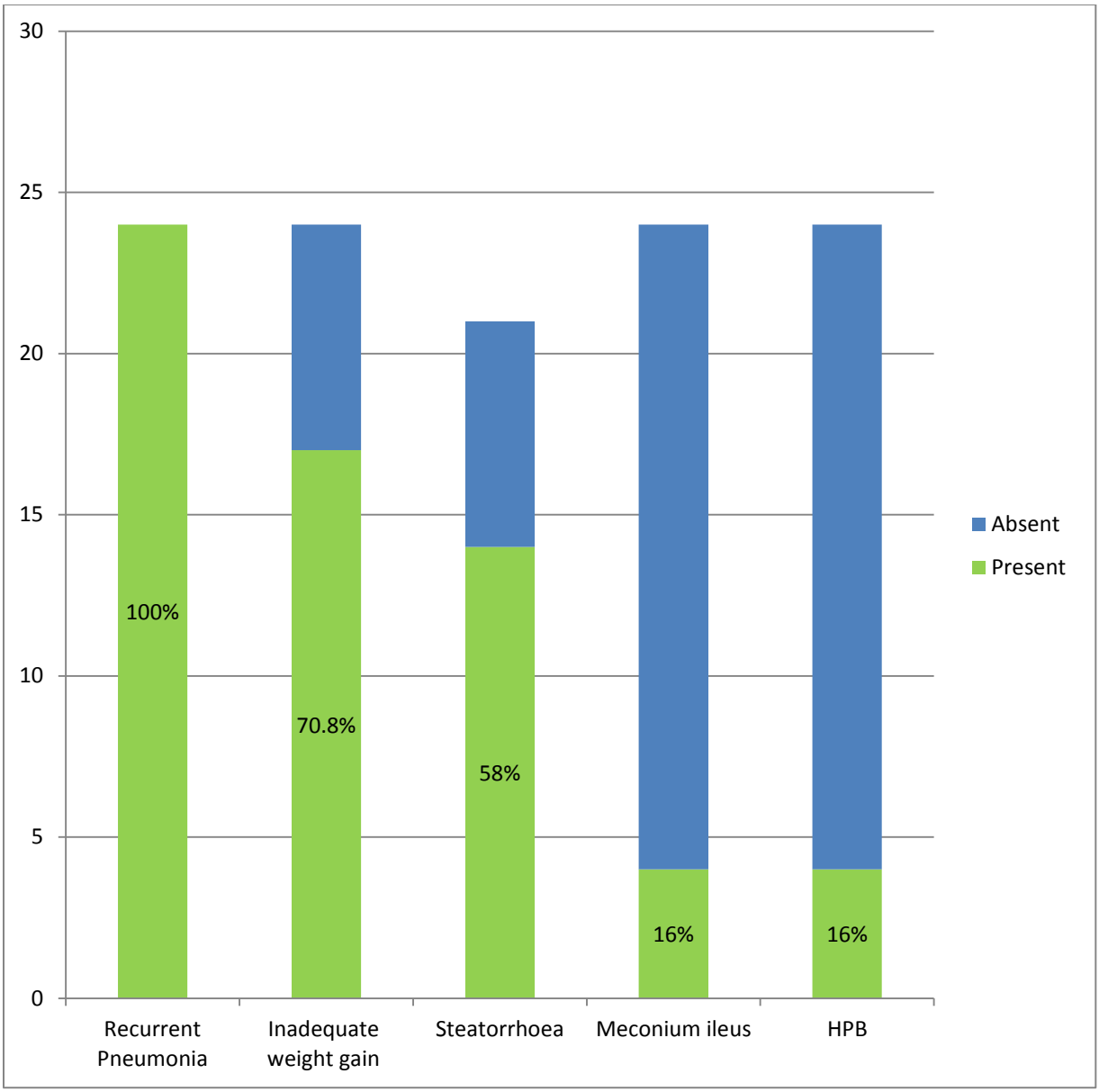


Fig 3.Age at the time of onset of symptoms

Majority of children were less than one year (54%) at the onset of symptoms ,median age being 8 months.



FTT- Failure to Thrive HPB- Hepatobiliary Disease DM- Diabetes Mellitus

Fig.4 Clinical symptom profile.

All children had recurrent respiratory infections, Other predominant complaints were inadequate weight gain and steatorrhoea.

History of salty taste to kiss was encountered in only one child.

Table1., Distribution of birth weight .

Birth weight range in grams	Number of Children
1500-2000	1 (4%)
2001-2500	4(16%)
2501-3000	12(50%)
3001-3500	7(29%)

50% children enrolled in the study had birth weight above 2500gm.Only one child had very low birth weight.

Genetic profile of CF patients (at least one mutation)

Table 2., Genetic profile of patients (number 13)

Mutation	Number of CF children
F508del	4(30%)
G551D	0
G542X	0
621G2T	0
M4740V	1(7%)
Mutation not detected	8(61%)
total	13(100%)

Mutation analysis results were available for 13 patients. F508 del was detected in 30%. Since mutation testing was done for only 4 common mutations and very few had complete sequencing of exons 10&11, we could not detect the mutation in 61%.

Symptoms of Pancreatic insufficiency

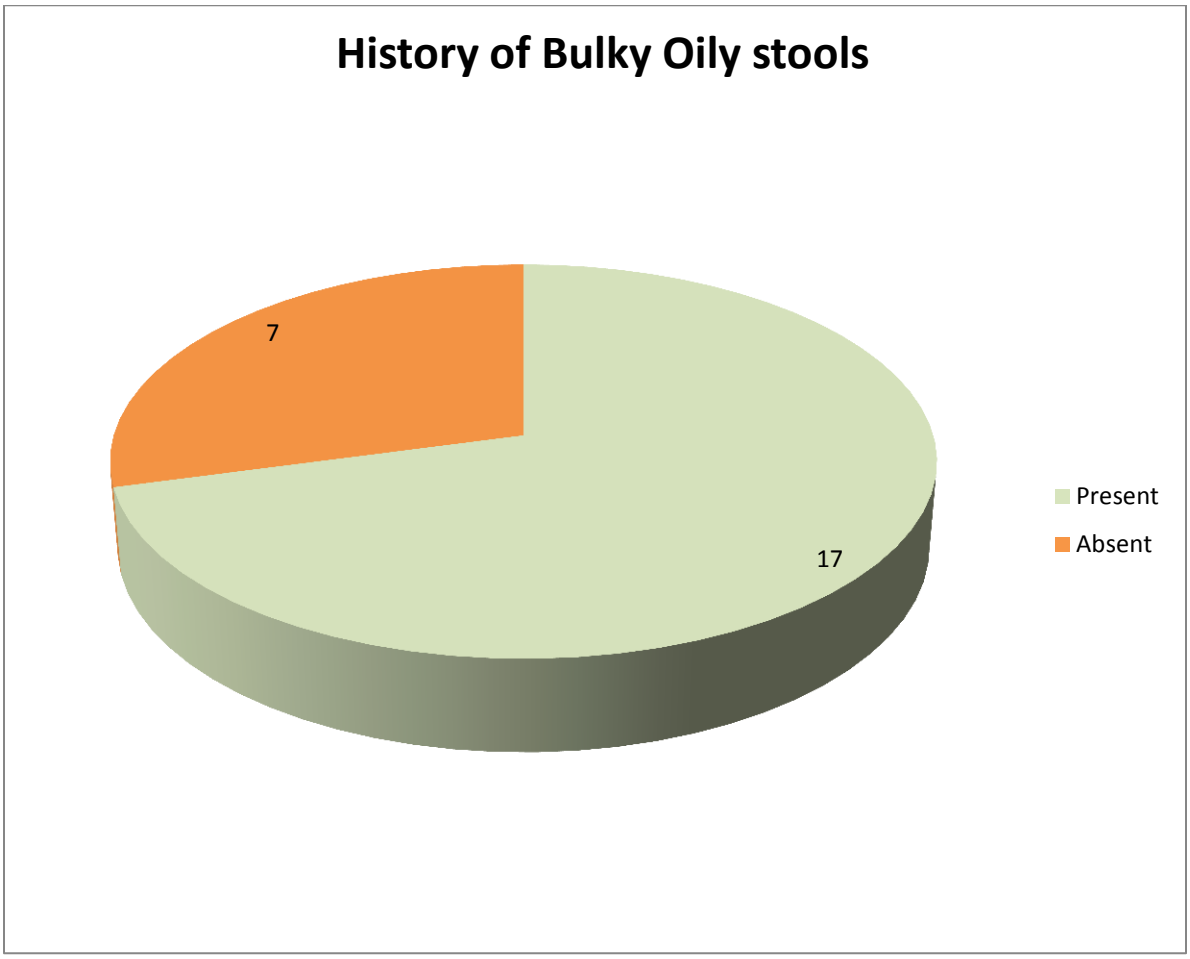


Fig 5. Children with CF who reported bulky oily stools.

Out of 24 children enrolled 70% reported bulky oily stools . However some of them were pancreatic sufficient on further testing.

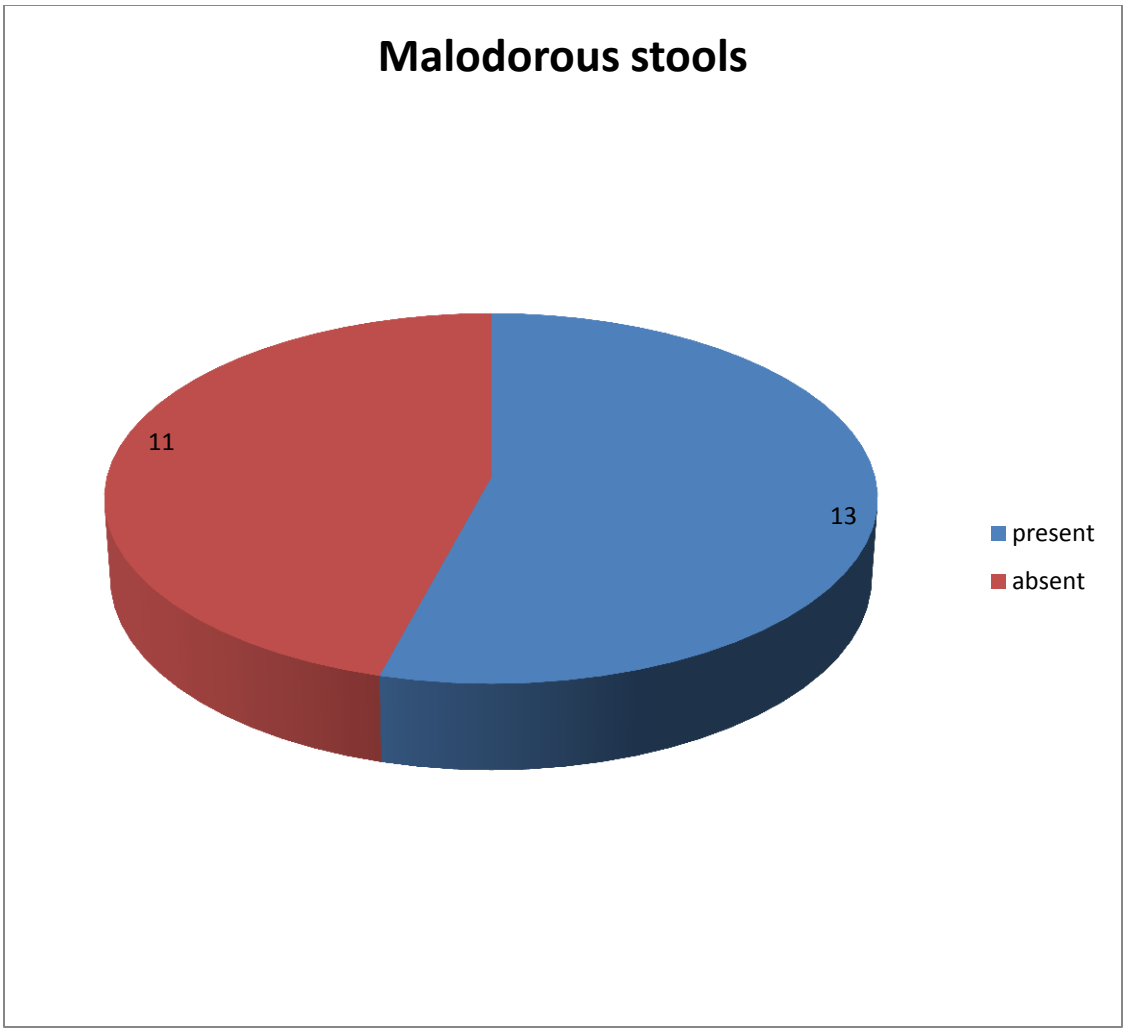


Fig 6.,History of malodorous stool

54% reported symptom of foul smelling stool.

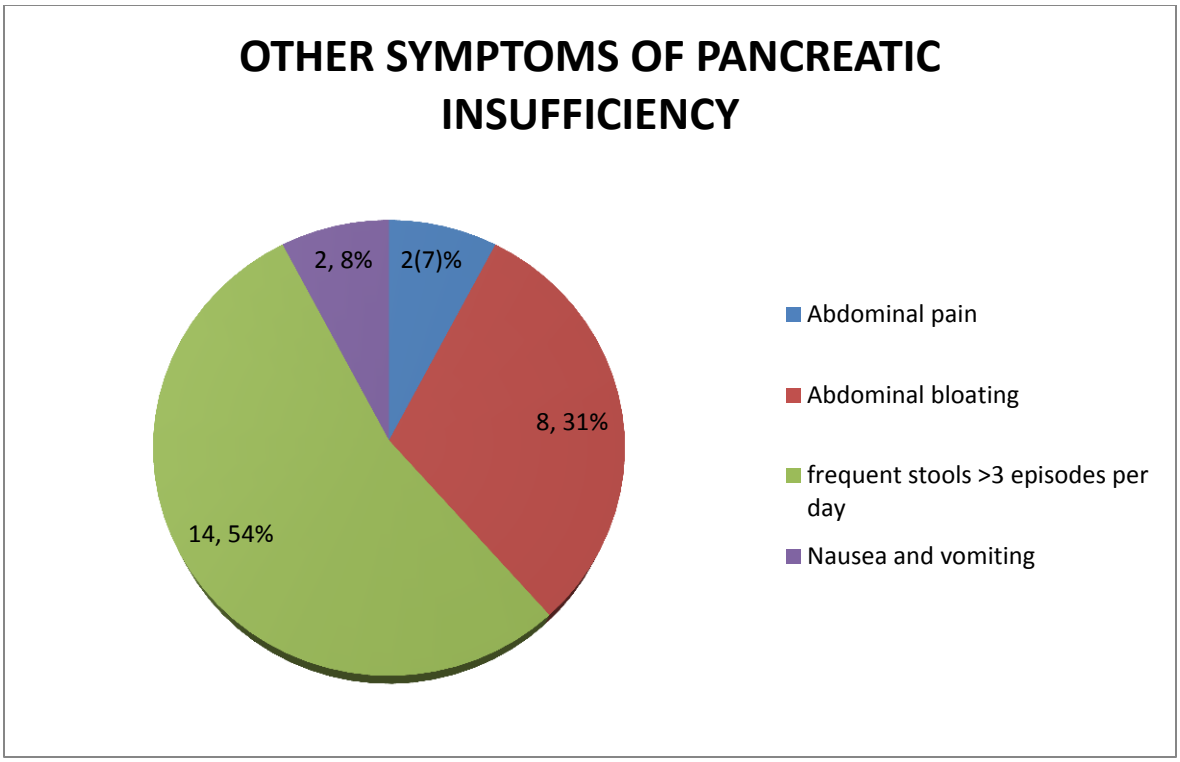


Fig .7. Symptoms of pancreatic insufficiency other than bulky, foul smelling stools.

Increased frequency of stools more than thrice a day was reported by majority of patients.

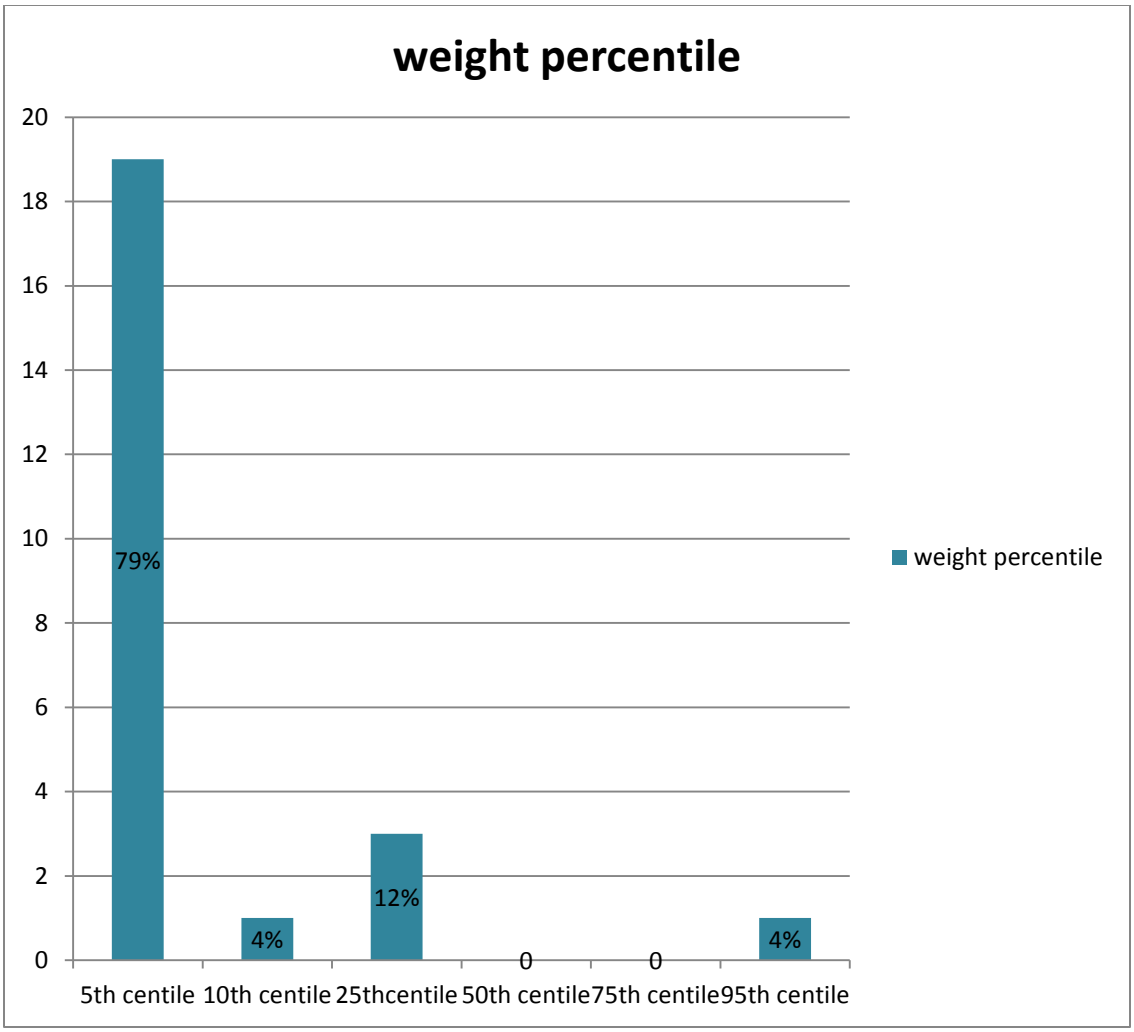


Fig.8. Weight centile distribution in children with CF.

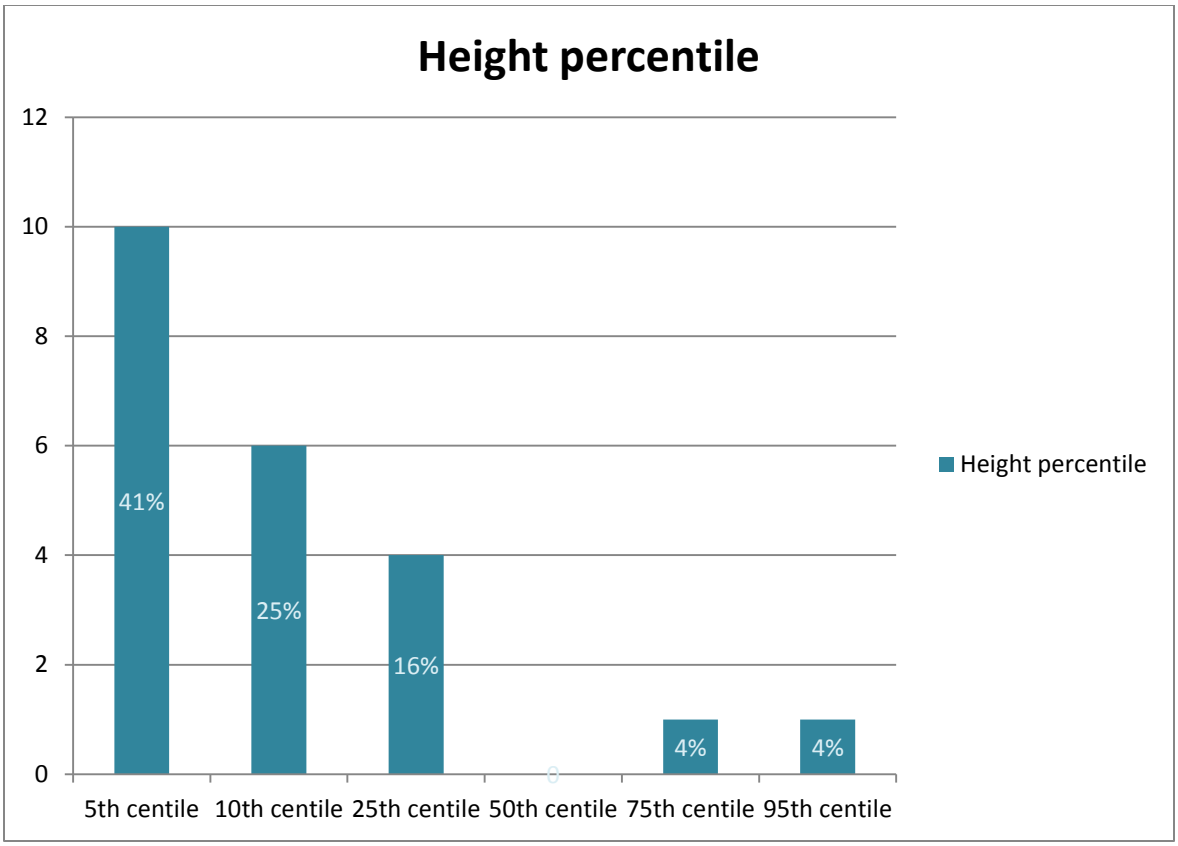


Fig.9. Height centile in children with CF

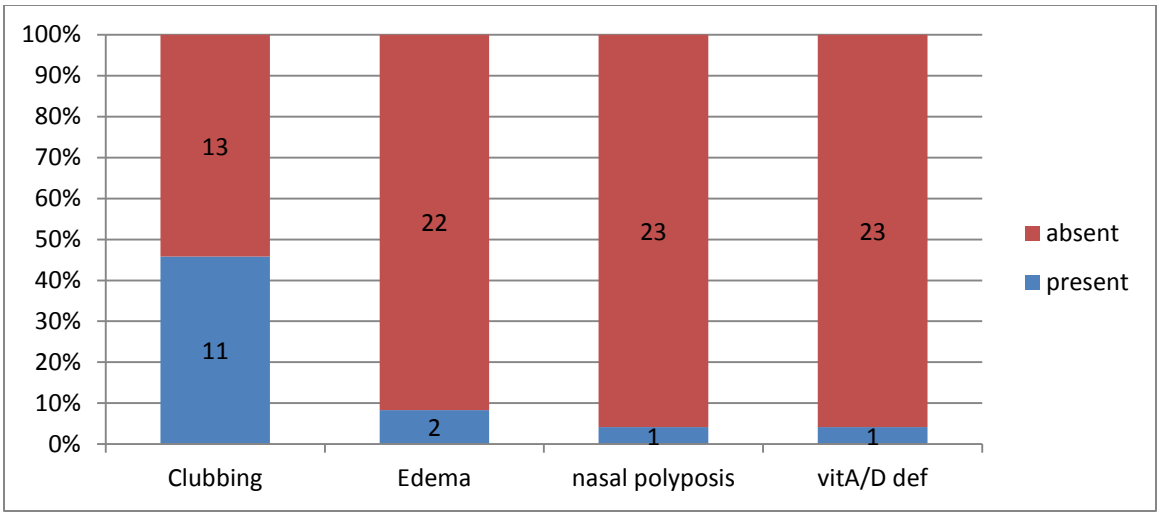


Fig.10. Signs of cystic fibrosis

Clubbing was observed in 65% of children and oedema 4%,while 1% had signs of fat soluble vitamin deficiency .

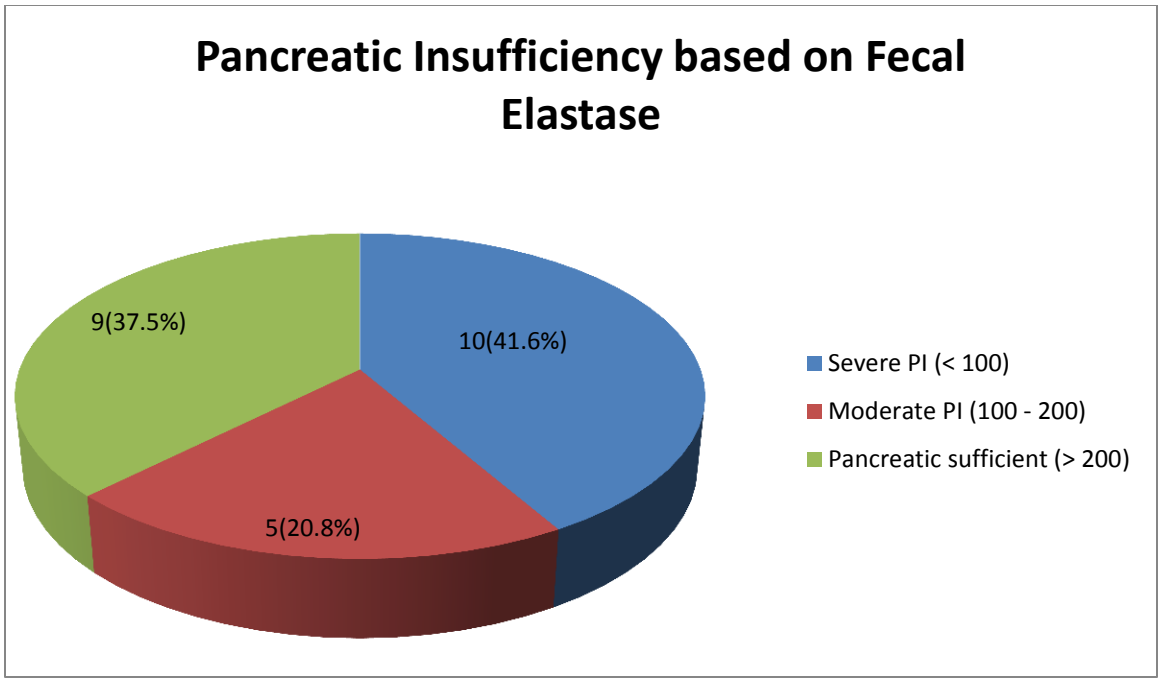


Fig.11. Severity of pancreatic Insufficiency .

Prevalence of pancreatic insufficiency was 62.4%,while

41.6% of children had severe pancreatic insufficiency

	number	percent
PS(FE>200)	9	37.5
Mod PI(FE 100-200)	5	20.8
SeverePI(FE<100)	10	41.6
TOTAL	24	100

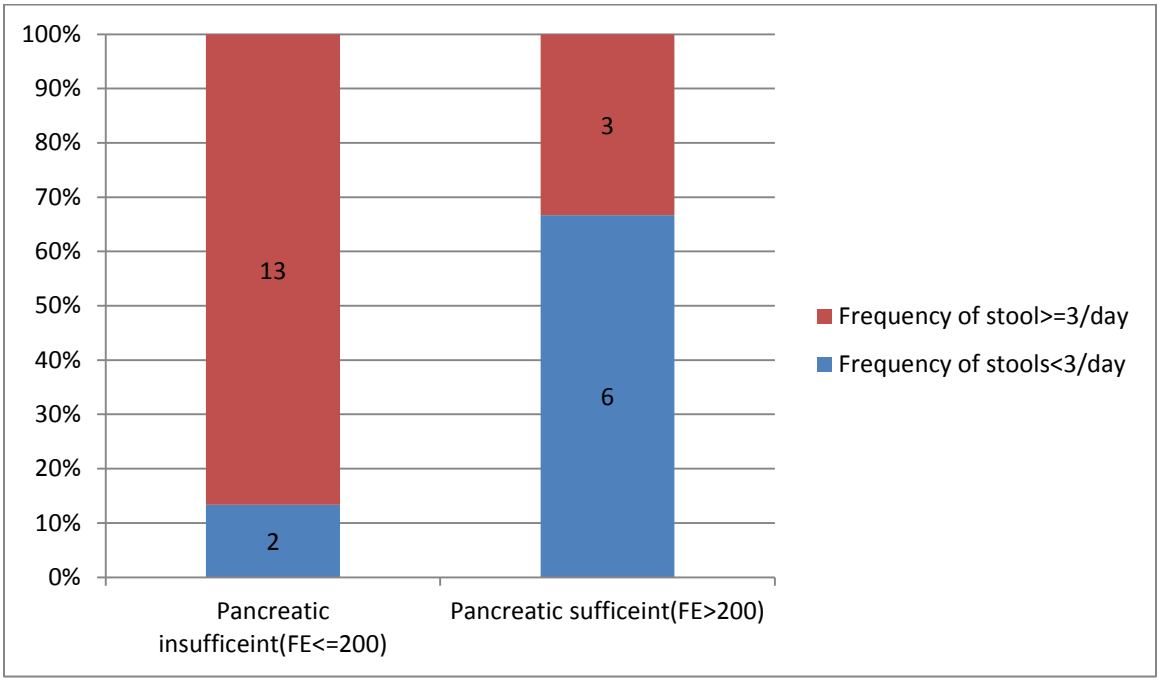
COMPARISION OF PANCREATIC INSUFFICIENT GROUP WITH PANCREATIC SUFFICIENT GROUP

Pancreatic insufficiency and ΔF508 mutation

Table 3 PI F508 del mutation

	ΔF508mutation present	**ΔF508mutation absent**	**TOTAL**
PI(FE<=200)	**4**	**9**	**15**
PS(FE> 200)	**0**	**9**	**9**
TOTAL	**4**	**18**	**24**

All Children in whom atleast one F508del was detected were pancreatic insufficient. Only I child had the mutation in homozygous state.



	Freq<3/day	Freq>=3/day	total	
PI(FE<200)	2(33.3%)	13(86.6%)	15(100%)	P=0.01
PS(FE>200)	6(66.6%)	3(33.33%)	9(100%)	
TOTAL	8	16	24	

Fig.13.,Frequency of stools vs pancreatic insufficiency.

History of passing more than 3 stools per day was significantly associated with pancreatic insufficiency.

Pancreatic Insufficiency vs Bulky Oily Stool

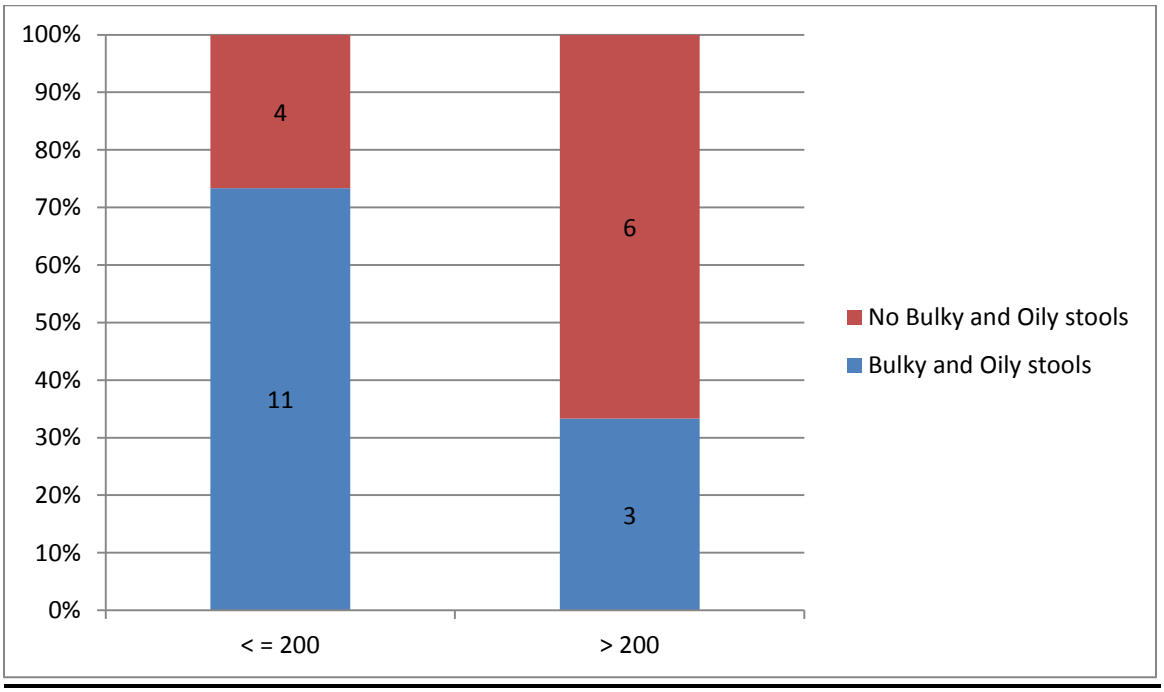


Fig.12.proportion of PI patients with bulky oily stools.

73.3% Pancreatic Insufficient children had bulky oily stools as a predominant symptom

	Bulky & Oily Stools	No Bulky & Oily Stools	Total	
PS (FE>200)	3 (33.3%)	6(66.7%)	9 (100%)	P=0.06
PI (FE<=200)	11(73.3%)	4 (26.7%)	15(100%)	

Pancreatic Insufficiency vs Foul Smelling Stool (FSS)

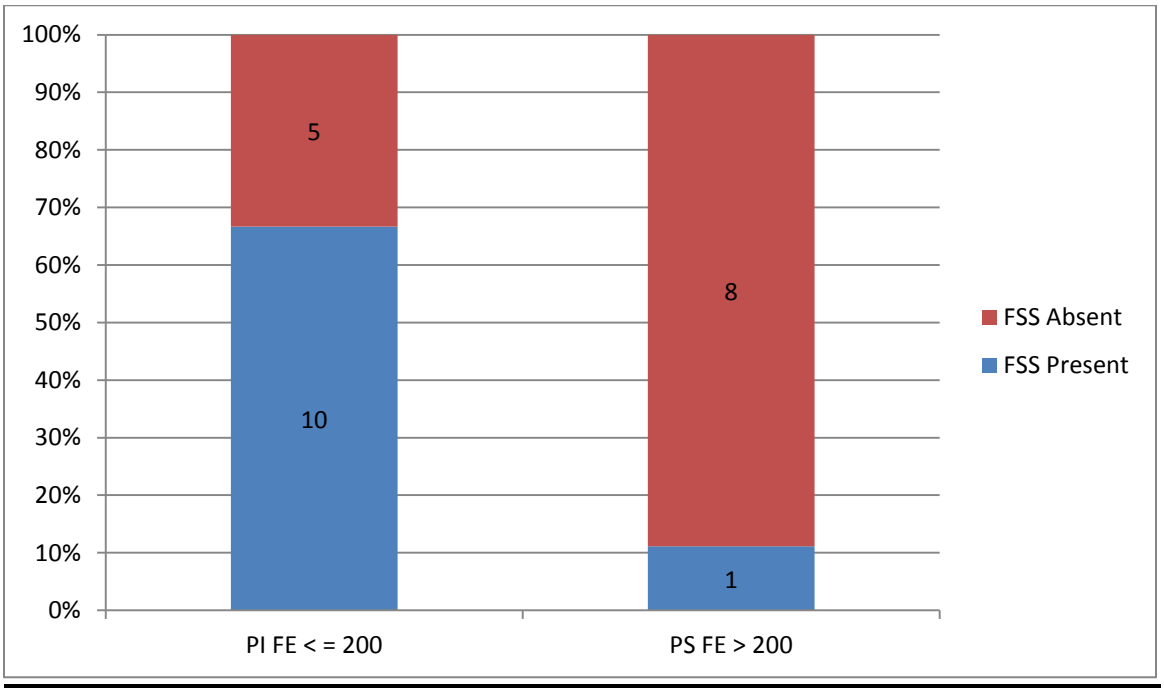


Fig.14. represents proportion of children with foul smelling stools among CF children

Statistically significant proportion of children had history of passing malodorous stool

	FSS present	FSS absent	Total	
PI FE <=200	10 (66.7%)	5 (33.3%)	15 (100%)	
PS FE > 200	1 (11.1%)	8 (88.9%)	9 (100 %)	P=0.02
Total	11	13	24	

Pancreatic Insufficiency vs Abdominal bloating(ABN)

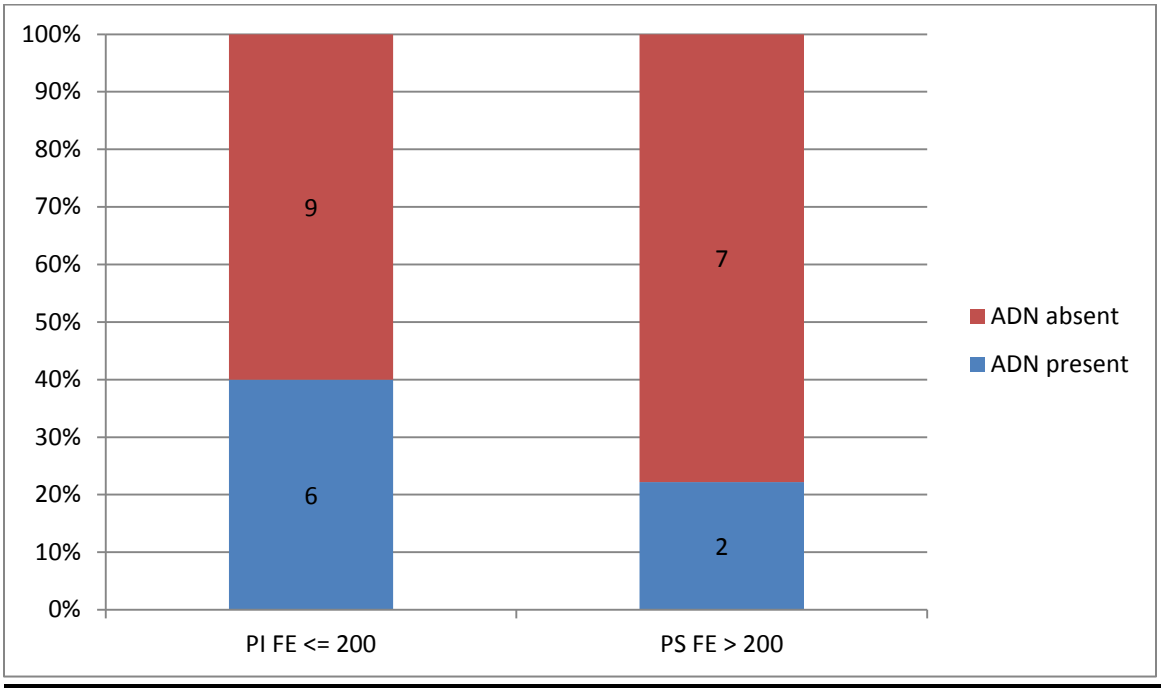


Fig.15. Pancreatic insufficient CF children with abdominal bloating.

There was no statistically significant difference in abdominal bloating symptom between PI and PS patients

	ABN Present	ABN absent	Total	
PI FE <=200	6 (40%)	9 (60%)	15 (100%)	
PS FE > 200	2 (22.2%)	7 (77.8%)	9 (100 %)	P=0.3
Total	8	15	24	

Pancreatic Insufficiency vs Abdominal Pain(ABP)

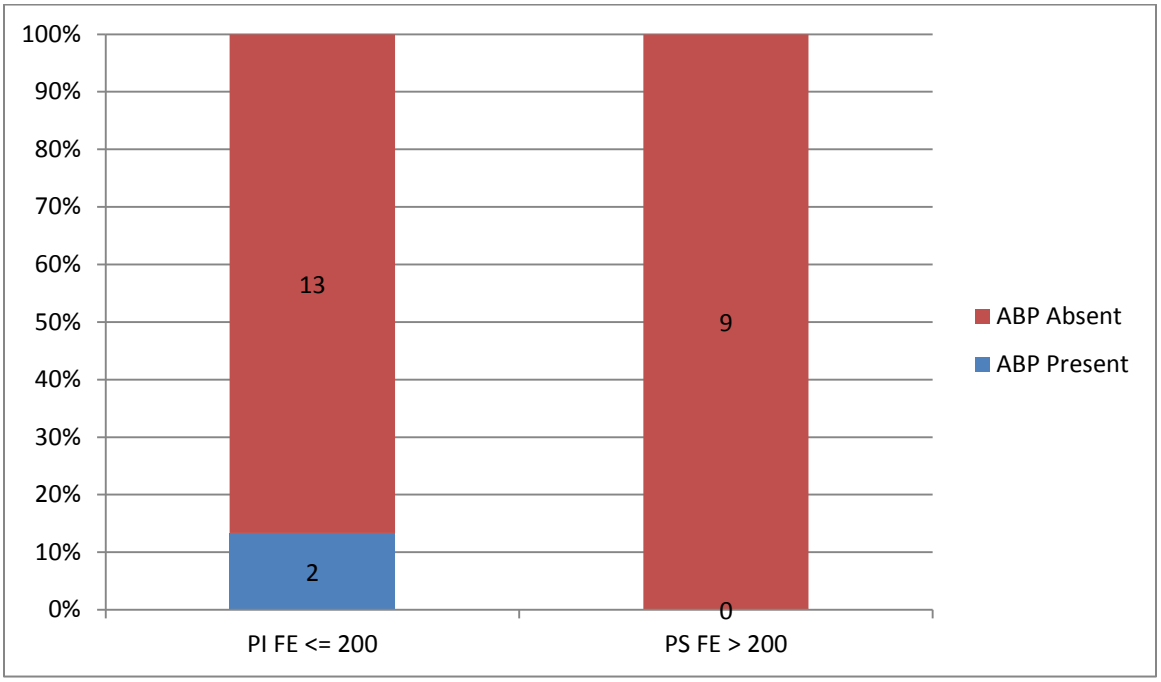


Fig.16. Proportion of children with abdominal pain

Abdominal pain with steatorrhoea was complained only by PI patients,

Pancreatic Insufficiency vs Inadequate Weight Gain(IWG)

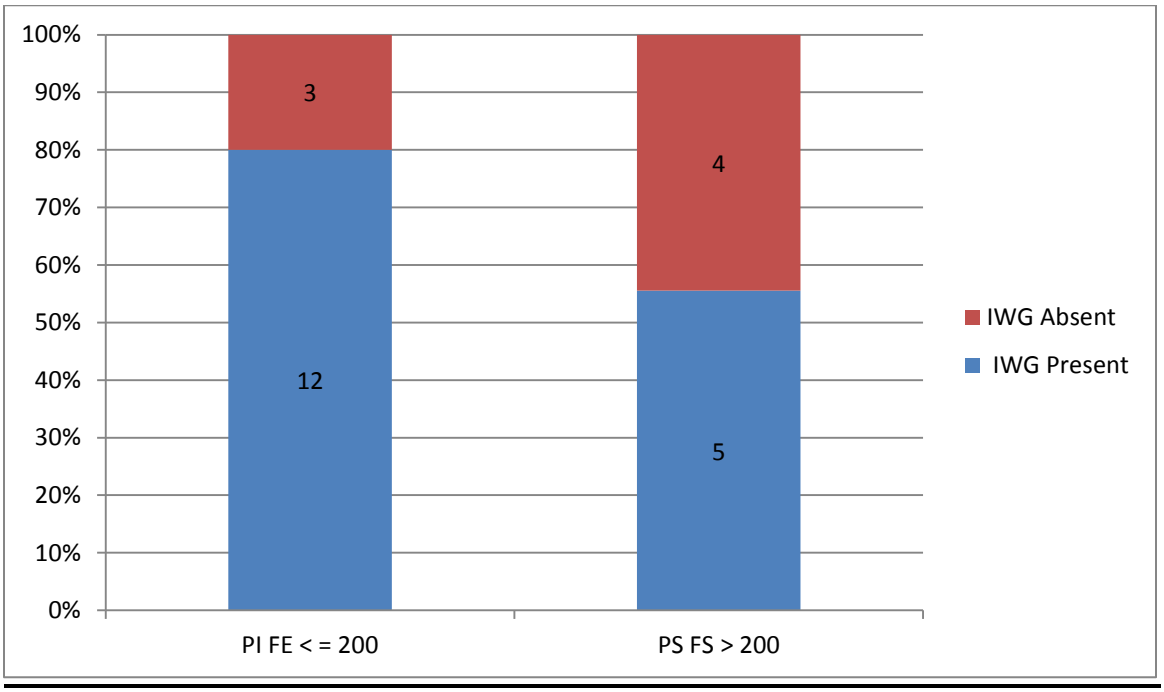


Fig.17., History of inadequate weight gain as reported by parents in PI and PS cystic fibrosis patients

There was no statistically significant difference between the 2 groups in terms of inadequate weight gain.

	Inadequate weight gain	Adequate weight gain	Total	
PI FE <=200	12 (80%)	3 (20%)	15 (100%)	P=0.2
PS FE > 200	5 (55.6%)	4 (44.4%)	9 (100 %)	

Pancreatic Insufficiency vs Weight Percentile(WP)

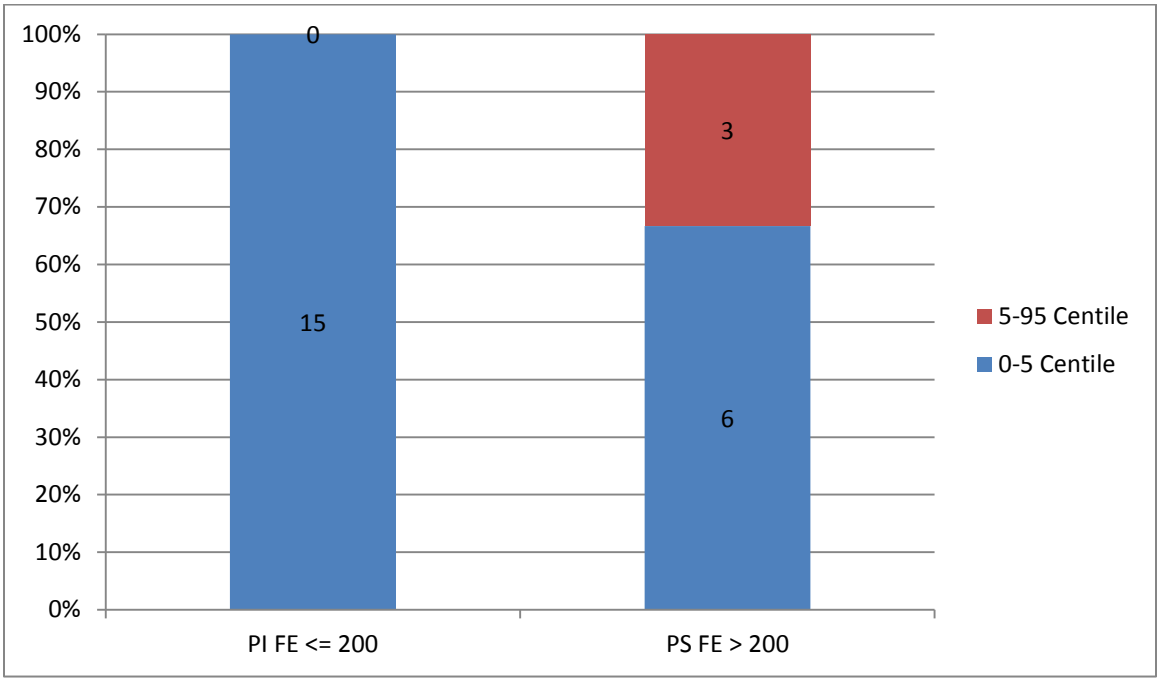


Fig 18., Exocrine pancreatic status and weight centile of CF patients.

Body weight of all PI children (100%) were below 5th centile on Agarwal chart.

	0 - 5 Centile	5 – 95 Centile	Total
PI FE <=200	15 (100%)	0 (0%)	15 (100%)
PS FE > 200	6 (66.7%)	3 (33.3%)	9 (100 %)
Total	21	3	24

Pancreatic Insufficiency vs Height Percentile(HP)

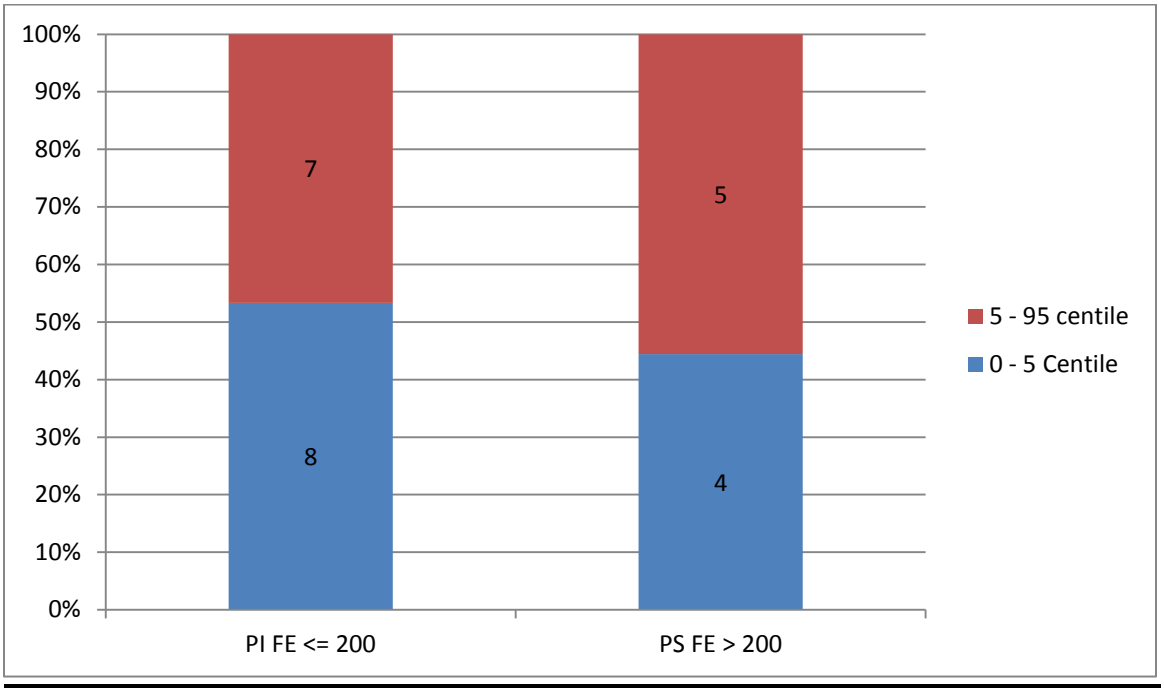


Fig.19. Exocrine pancreatic status and height centile

There was no significant difference between PI and PS patients in height centile.

	0 - 5 Centile	5 – 95 Centile	Total	
PI FE <=200	8 (53.3%)	7 (46.6%)	15 (100%)	
PS FE > 200	4 (44.4%)	5 (55.6%)	9 (100 %)	P=0.09
Total	12	12	24	

Pancreatic Insufficiency vs Age at onset of symptoms

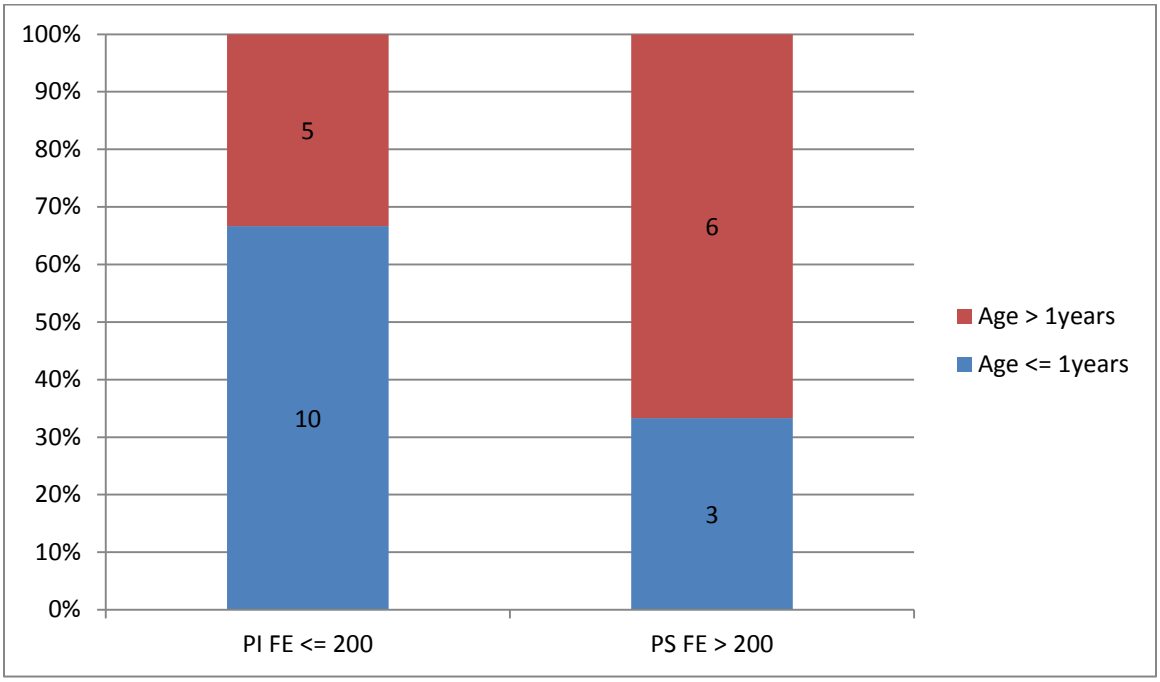


Fig.20. represents relationship of PI with age at diagnosis

66.6% of children were less than 1 year during the onset of symptoms with median age being 8 months.

	Age <= 1 year	Age > 1 year	Total	
PI FE <=200	10(66.6%)	5(33.3%)	15 (100%)	
PS FE > 200	3(33.3%)	6(66.6%)	9 (100 %)	P=0.2
Total	13	11	24	

Pancreatic Insufficiency vs Birth weight (BW)

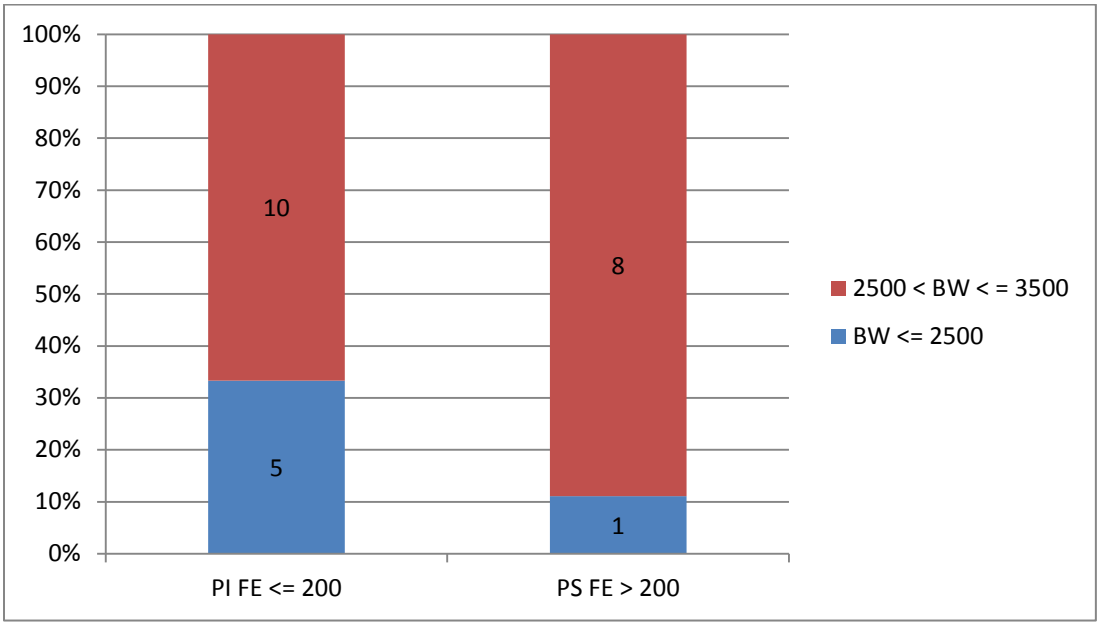


Fig.21., represents relationship of PI with birth weight of CF children

33.3% of children with pancreatic insufficiency were of low birth weight i.e. BW< 2500 grams.

	BW <= 2500 (in grams)	**2500 < BW <=3500 (in grams)**	**Total**	P=0.3
PI FE <=200	5 (33.3%)	10 (66.6%)	15 (100%)	
PS FE > 200	1 (11.1%)	8 (88.9%)	9 (100 %)	

Pancreatic Insufficiency vs Delayed Passage of Meconium(DPM)

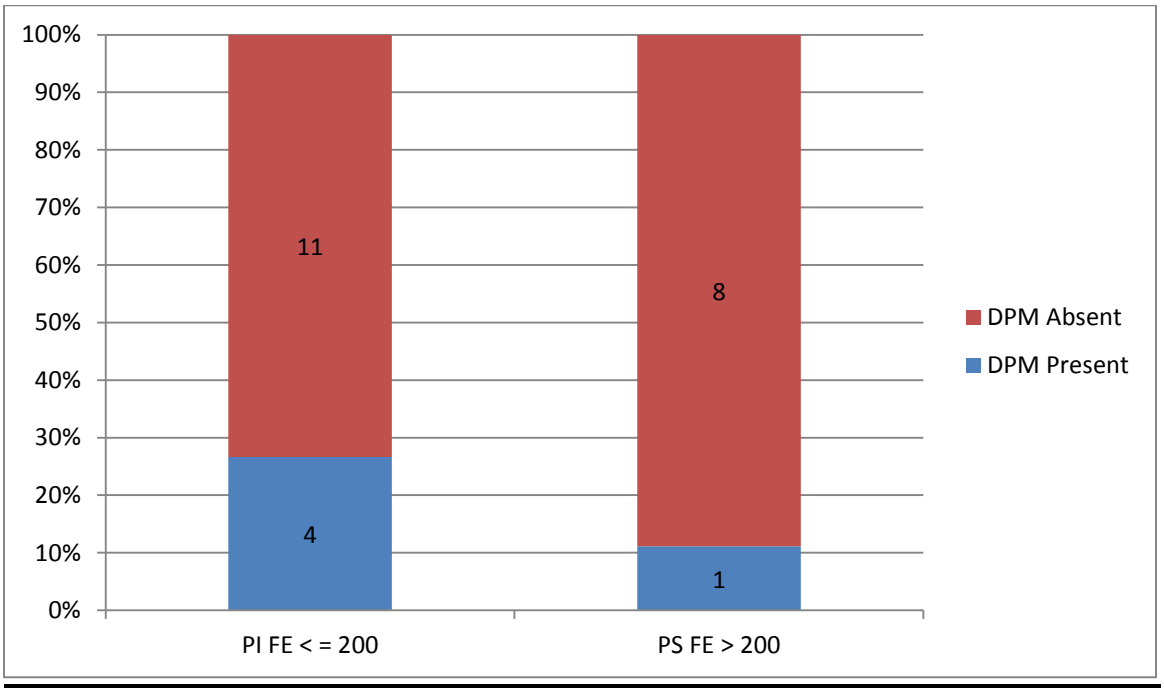


Fig.22.,Exocrine pancreatic status and history of delayed Passage of Meconium (DPM) at birth

26.7% historically had delayed passage of meconium at birth. One among the four patients had meconium ileus requiring surgery in neonatal period.

	DPM Present	DPM absent	Total	
PI FE <=200	4 (26.7%)	11 (73.3%)	15 (100%)	P=0.6
PS FE > 200	0 (0%)	9(100%)	9 (100 %)	

Pancreatic Insufficiency vs Fat globules on stool microscopy (FG)

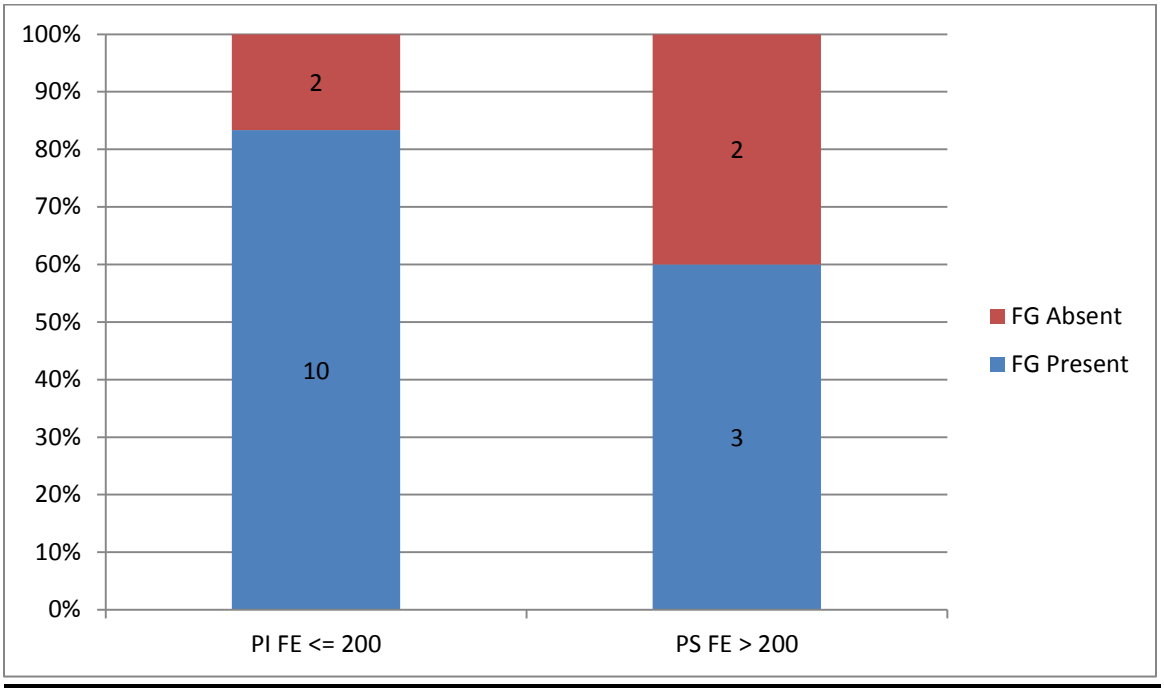


Fig.23. PI and stool microscopy for fat globules.

83.3% of PI children had numerous fat globules on stool microscopy but there was no statistically significant difference from those who were pancreatic sufficient.

	Fat Globules Present	Fat Globules Absent	Total	
PI FE <=200	10 (83.3%)	2 (16.7%)	12 (100%)	P=0.5
PS FE > 200	3 (60%)	2 (40%)	5 (100 %)	

Bronchiectasis was assessed by imaging. All patients had chest X ray and 11 patients had High resolution CT of chest. Bronchiectasis could be diagnosed in 55.5% PS and 44% PI patients with no statistical difference between the 2 groups. As many patients were infants bronchiectasis may not have developed and this assessment may not have been optimum.

Relation between pancreatic insufficiency and clinical severity of CF

Deaths and long term oxygen therapy(LTOT)

	death	**LTOT**
PI(FE<=200)	**2**	**2**
PS(FE>200)	**0**	**0**

Table 4 Exocrine pancreatic status and poor outcome of CF

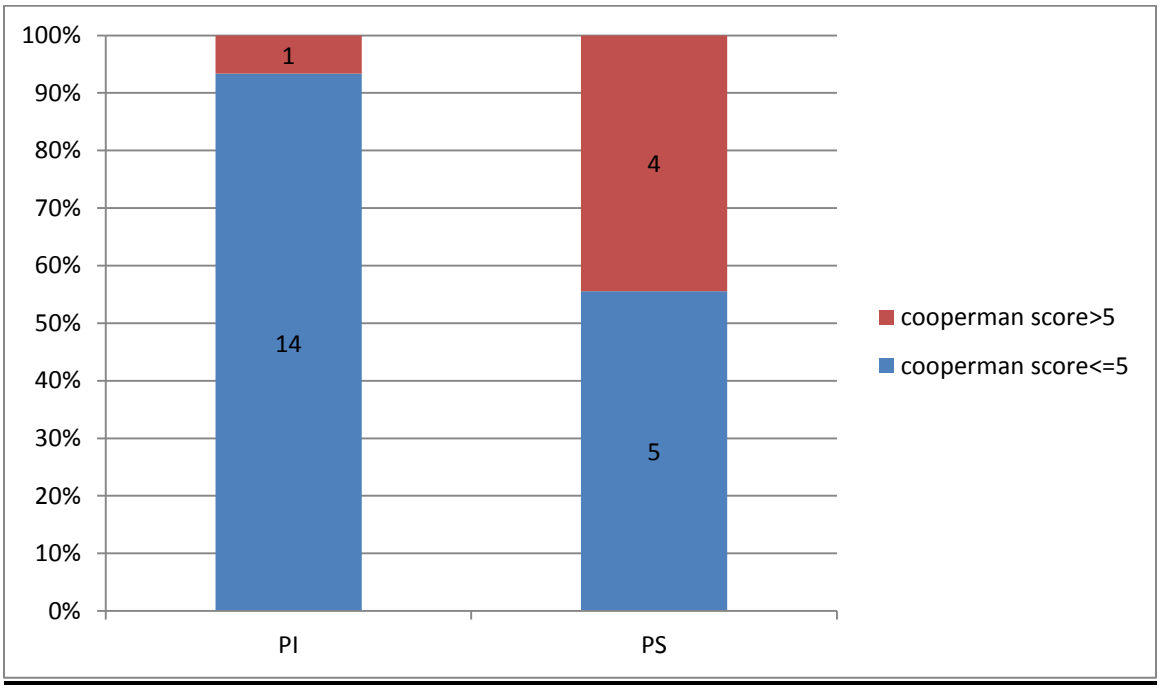
2 children in the pancreatic insufficient group were on long term home oxygen and died during the study .Pancreatic sufficient children did not need oxygen at home and there was no reported mortality.

Table5 CF related diabetes mellitus (CFRDM) use total and %

	CFRDM present	CFRDM absent
PI(FE<=200)	**2**	**13**
PS(FE>200)	**0**	**9**

2 patients in the pancreatic insufficient group required insulin therapy .

Fig 23 Severity of disease based on Cooperman score (annexure 7)and its relation to pancreatic insufficiency



Using Cooperman score(Annexure 7) ranging from 0-10 (0 being worst and 10 being best) 94% of Pancreatic insufficient children had lower scores and 80% pancreatic sufficient group had higher score. This difference was statistically different p=0.04.

Pancreatic insufficiency vs pseudomonas colonisation

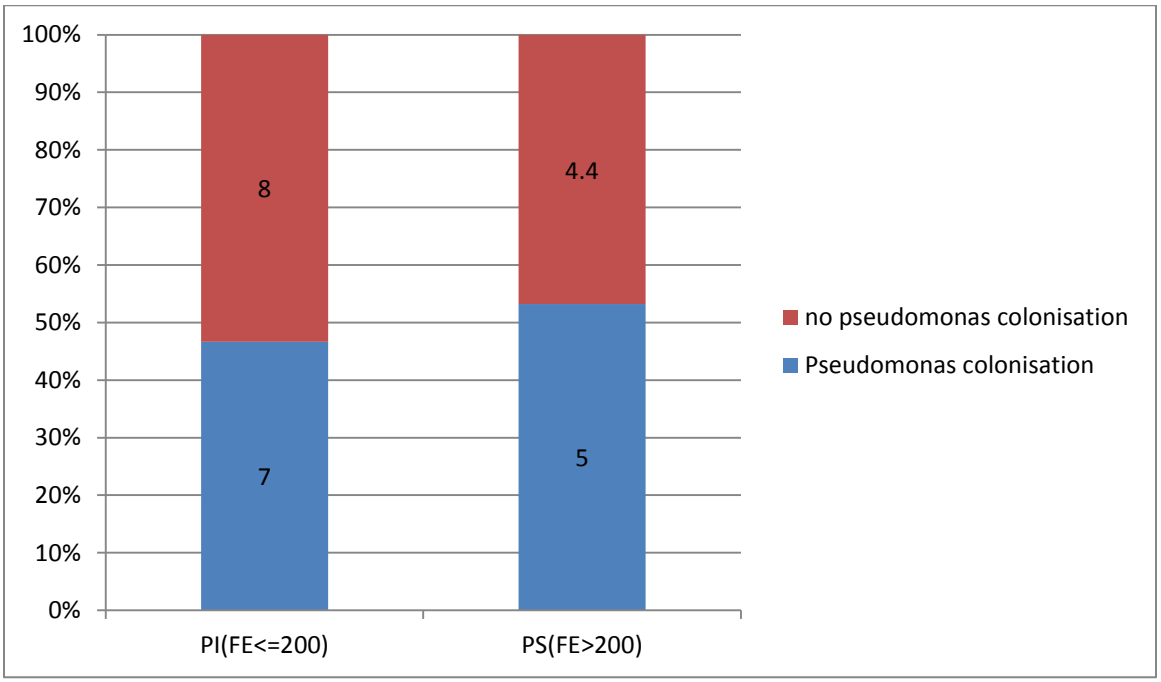


Fig24,Pancreatic insufficiency and pseudomonas colonisation.

Pseudomonas colonisation marks the decline of pulmonary function in CF patients. There was no statistical difference between PI and PS patients in presence of pseudomonas aeruginosa in airway secretions.

	Pseudo.colonisation	No.pseudo colonisation	Total	
PI(FE<=200)	7(46.6%)	8(53.3%)	15(100%)	P=0.2
PS(FE>200)	5(55.5%)	4(45.5%)	9(100%)	
Total	12	12	24	

Pancreatic insufficiency and high sweat electrolyte concentration

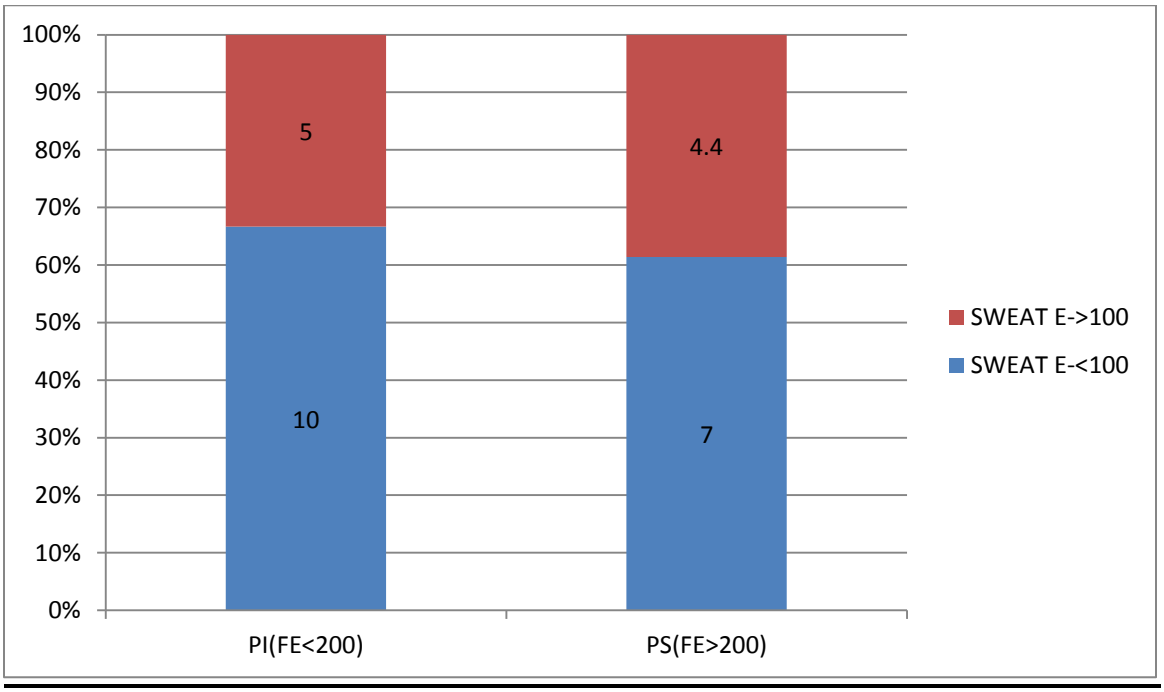


Fig 25., Exocrine pancreatic status and sweat electrolyte level.

Highly elevated sweat electrolyte level was seen in both groups equally.

It was noted that in the pancreatic insufficient group majority (66%) had values < 100meq/lit.

	Sweat e-<100	Sweat e->=100		
PI(FE<200)	10(66.6%)	5(33.33%)	15(100%)	
PS(FE>200)	7(87.5%)	1(12.5%)	8(100%)	P=0.2
Total	17	6	23	

Development of a clinical score to predict pancreatic insufficiency

Table 6., Bivariate analysis for potential variables using chi square test with Yates correction.

Variables	P value	Significance(RR)	95% CI
Foul smelling stool	0.02	16	1.5-166
Age at presentation < 5years	0.01	13	1.7-99.3
Frequency of stool>=3/day	0.01	13	1.7-99.3
Inadequate weight gain	0.2	3.2	0.5-19.8
Bulky oily stool	0.06	5.5	0.9-33.1
Abdominal distension	0.3	2.33	0.3-15.3
Height centile	0.14	4	0.6-25.9
clubbing	0.08	5.2	0.8-34.4
Presence of fat globules	0.3	3.33	0.3-34.8

Table 6 . represents results of the multivariate regression analysis for significant variables

Significant variables	**P value**	**Relative Risk**	**95% CI**
Foul smelling stools	**0.15**	**9.8**	**(0.42-226.2)**
Frequency of stools >=3 per day	**0.56**	**2.2**	**(0.14-34.5)**
Age of child at diagnosis	**0.06**	**10.7**	**(0.8-139.9)**

DISCUSSION

DISCUSSION

Cystic fibrosis ,once considered a disease of the caucasians is increasingly being recognised and diagnosed in Indian children. While we do not have exact data on prevalence certain estimates have been made. Even if the incidence is as low as 1 in 40000 - as estimate based on migrant population data in the US- considering the vast population in India, children with cystic fibrosis living in India may outnumber those in many western countries.

One of the main problems in India is under diagnosis of the condition even when classical features are present . Delayed diagnosis leads to increase in morbidity, wasteful expenditure on incorrect and unnecessary treatment and rapid progression of the disease. Cystic fibrosis is the commonest cause of pancreatic insufficiency. However many paediatricians and even gastroenterologists do not evaluate the child adequately to rule out cystic fibrosis, even when they recognise pancreatic insufficiency/ fat mal absorption.

This prospective observational study was carried out in a tertiary care centre to addresses some of the questions about pancreatic insufficiency in CF children in India. Prospective nature of the study provided us an opportunity to collect good quality data on history and examination of these patients. Consecutive patients utilising the CF clinical service of Paediatric department who satisfied the inclusion criteria were recruited to the study thereby avoiding a selection bias.

Through this study we attempted to study manifestations of pancreatic insufficiency in our cystic fibrosis population, to find out the proportion of children who are pancreatic

insufficient (PI) using a sensitive and specific test - Faecal elastase. It was also our objective to compare the differences between the pancreatic sufficient (PS) and pancreatic insufficient (PI) groups in terms of presenting features , severity of lung involvement and other CF manifestations.

24 consecutive CF patients who attended our paediatric service areas during the study period were included in the study. This group was comparable to other case series reported from other parts of India in terms of demographic data ,disease presentation , anthropometric indices and disease severity.

75% of this series were boys. Most case series from India show a male predominance(16) . Globally girls with CF are noted to have a worse outcome and life expectancy is lower for females (82) .Since this is not a study planned to assess the survival benefit of any sex we cannot claim that the higher proportion of boys in this study is due to better survival of males. It may be just representing the health seeking behaviour of the population where male children are provided better health care than girls.

Only 1 child was born with very low birth weight and majority (79%)had satisfactory birth weight above 2500gm. Malnutrition sets in after the neonatal period as shown by research in developed counties comparing data before and after initiation of neonatal screening programme(62).

In keeping with observation from other centres ,we also noted a significant delay in making the diagnosis. While age at diagnosis for our population was 76 months, studies done at New Delhi(16) and Chandigarh,(1) age at the time of diagnosis were 54 and 57 months, respectively.

The average delay in diagnosis was 47 months from the onset of symptoms This is similar to the study done by Kabra et al. where the average gap between the onset of symptoms and diagnosis was 44 months.. This information obtained from the medical records/history by parents may not be exact and there is bound to be variability. Even then,it is evident that there is low index of suspicion for the disease in India and need there is need increased awareness for same(16).

50% of the subjects had pseudomonas colonization of airways at the time of recruitment.. This is similar to Study done in AIIMS that showed 50% patients already had pseudomonas colonization in their airway at the time of diagnosis(16). Study done in Kashmir showed 33.3% of patients colonized with pseudomonas aeruginosa and 33.3% colonized with Staphylococcus aureus(1).

In our series of cystic fibrosis children, predominant clinical features at presentation are failure to gain weight (80%), recurrent respiratory infections (100%) and history suggestive of mal absorption similar to the data from PGIMER Chandigarh which

showed statistics of 94% failure to thrive and 74% recurrent or persistent respiratory tract infection at the time of presentation.

Blackman et al (29) reported an incidence of 7-13% meconium using the data collected by Twin and Study in United states in sibilings of CF children, monozygotic and dizygotic twin.

There was only 1 child who had meconium ileus surgery in our cohort. History of meconium ileus equivalents i.e., delayed passage of meconium was reported in 16% of patients enrolled in the study. These figures are not comparable as we would have come across only CF children who survived the neonatal period and our figures does not represent the true meconium ileus prevalence among CF patients,in a developing country.

Though salty taste to kiss is taught to medical students as common clinical feature of the condition, only one family reported this symptom. Positive family history of confirmed or suspected CF was present in only 8%..Other classic features like rectal prolapse (4%,) nasal polyposis (4%) and pseudobarter (4%) presentation was seen rarely in this group. Similar observation was made by researchers elsewhere also.(24,31).

From the above discussion it is evident that the main presenting features are nonspecific like recurrent respiratory infections and failure to thrive and the more specific features of CF are rarely encountered. However pancreatic insuffciency and fat mal absorption which are rare in childhood , when present should alert one about the possibility of CF .This is a pragmatic approach for developing countries like India. It is important to know

what percentage of CF patients have pancreatic insufficiency in India and that was the primary objective of our study.

Best method to answer the above question will be to identify the total number of CF patients in a sample population by neonatal screening and then find out how many of them have PI. With no neonatal screening programme in place that method is impractical. Hence we resorted to our methodology as a hospital based study. Children who get referred to a tertiary care hospital belong to the severe end of the phenotypic spectrum of CF, who have mutations causing severe illness .Hence pancreatic insufficiency will be overrepresented in this group. In our study the proportion of Pancreatic insufficiency(Fig 11,) was 62.5% (95% CI-42.7-82.3).This is slightly lower than the Indian study done by Kabra et al (19) where the prevalence of malabsorption was reported as 80% and studies done in NewYork classify pancreatic insufficient people at CF centres did show a prevalence of 85%(39).

In an attempt to find the features that define the clinical profile of pancreatic deficient patients and differentiate them from the PS patients , we compared the 2 groups in terms of certain features as below. In this series history of foul smelling stools and increased frequency of stools showed a statistically significant association with pancreatic insufficiency. Although 73% of children with PI gave history of passing bulky oily stools compared to33.3 % in PS patients(Fig 12.,) this did not reach statistical significance. Similarly history of abdominal pain and bloating was present in 40% of PI and 13% of PS patients. Routine Indian diet has less fat content than western diet. Hence significant steatorrhoea symptoms like bulky ,oily stools may not be reported in many PI patients. In subsequent studies we may need to rephrase the question and ask if

these are present when child consumes a fatty meal. Other studies from India did not address or report any differences in symptomatology.

Though overall there was a unacceptable delay in diagnosis for most patients,in pancreatic insufficient patients diagnosis was made earlier . They were more likely to have severe lung disease, need for home oxygen use ,relatively worse growth and more likely to have pseudomonas colonisation compared to their PS counterparts. Though these findings were not statistically significant in the present study ,it is quite likely that a study with a higher sample size will show statistical significance. Two patients who died during the study were pancreatic insufficient. As expected 2 patients who are on insulin therapy for endocrine pancreatic insufficiency (insulin deficeincy) also have exocrine deficiency.

Pancreatic insufficiency is known to correlate with malnutrition and therefore poor lung function(58) .In our study we found that only 40% of PI children had associated bronchiectasis.This can be explained as many of the subjects included were very young in whom bronchiectasis has not yet developed. As this was not the primary objective of the study we did not do HRCT which is the best modality for diagnosis of bronchiectasis in all patients . However we used a clinical score Cooperman score (annexure 7) which has been shown to correlate very well with lung function(80) and found that PI patient had a lower (worse) score compared to their PS counterparts .This difference was statistically significant.

In our study 66.6%(Fig 25) of children with Pancreatic insufficiency had sweat electrolyte concentrations less than 100.This observation has been noted in the literature where low sweat chloride concentrations were observed in children with PI due to hypoproteinemia causing edema (83).

Development of a clinical score to predict pancreatic insufficiency was one of the secondary objectives of our study. Literature review showed that though there are many clinical scores like Shwachman , NIH,Score by Kanga et al(79) to predict severity, prognosis etc there are none to predict PI. This may not be a priority in developing countries where tests for pancreatic function can be done easily. Pancreatic Insuffiency Prevalence (PIP) score predicts PI when genetic mutation is known(81) However exact determination of the mutation in a given patient is expensive and impossible for majority in a developing country.. Hence a clinical score will be a valuable tool for the clinician in India.

We proposed to develop the score by identifying the clinical charecteristics which correlate with PI by bivaiate logistic regression and then computing a score ,by multivariate logistic regression and plotting ROC curve sing Relatve risks of independent significant variables. However we were able to recruit only 24 patients to our study applying the inclusion criteria. This number was inadequate for the development of a score .Neverthless we identified 2 variables namely frequency of stools(>=3/day), foul smelling stools as having a high RR for being associated with PI. In addition few others like presence of bulky oily stool, abdominal bloating may be

candidate variables which can be used in the score. We propose to continue this study for an extended duration till we have enough number of subjects for this purpose.

.

.

.

.

.

LIMITATIONS

Limitations:

1.This prospective observational study was done on small sample of patients attending a tertiary care centre. Due to referral bias pancreatic insufficiency may be over represented in this group.

2. As the duration of study was very short ,sample size could not be reached. We could not meet one of the proposed secondary objectives namely development of a clinical sore to predict PI

3.As few of these patients were already on pancreatic supplements we could not do microscopic examination of stool for fat droplets in them. This simple lab test may have the potential to be a screening test and could be used as a variable for clinical score development.

4.Investigations like high resolution chest imaging, mutation analysis were not done for all patients due to financial constraints. Hence comparison between the pancreatic sufficient and insufficient groups with regard to severity of the lung disease and genetic profile is of limited value.There could be some inaccuracies in symptom reporting by parents as this is a chronic disease .

.

CONCLUSIONS

Conclusions

1. In this prospective study on 24 cystic fibrosis children who attended a tertiary care centre in South India during the study period November 2013 to August 2014, 64% (CI 42.7-82.3) had pancreatic insufficiency as diagnosed by low faecal elastase level of <200 microgram/gram . 41.6% of them had very severe pancreatic insufficiency with faecal elastase level < 100 microgram/gram

2. If only history suggestive of steatorrhoea was used for identifying children with pancreatic insufficiency only 45 % of these CF children would have been diagnosed as pancreatic insufficient. Using faecal elastase test we were able to identify another 19% and offer pancreatic enzyme replacement..

3..Clinical profile of these 24 patients is similar to that described from other parts of the world and India with predominant manifestations of recurrent respiratory infections (100%) ,)failure to thrive (79 %) and fat malabsobtion .Other classic features of cystic fibrosis like meconium ileus, rectal prolapse, nasal polyposis were seen in only a minority of patients.In this cohort median age of onset of symptoms is 8 months while that of age at diagnosis is 76 months.

4. All four children in whom F508del mutation was detected were pancreatic insufficient. Only 1 child in this group was homozygous for the mutation.

5. During the study period 2 patients from the pancreatic insufficient group died due to severe lung disease. Both were less than 2 years of age and were on oxygen therapy at home.

6.. Pancreatic insufficient group had lower (worse) Cooperman score for clinical severity which was statistically significant. The mean score of patients with pancreatic insufficiency was 2 .However there was no statistically significant difference between the 2 groups in severity of malnutrition or airway colonisation with pseudomonas aeruginosa.

7.By bivariate logistic regression and multivariate analysis 2 variables were identified for computing a clinical score for prediction of pancreatic insufficiency. They were history of >/= 3 stools /day and history of malodourous stools .However due to the small number of cases we could not develop the score.

RECOMMENDATIONS

Recommendations

1) Increase awareness among medical professional in India about common manifestations of cystic fibrosis in children. Screening for cystic fibrosis should be done when fat mal absorption features are present along with recurrent respiratory infections

.

2) Creation of a cystic fibrosis registry of CF patients of Indian origin is highly recommended as each centre has only limited number of cases.. Research using such a large database will provide answers to questions regarding early identification and optimal management of these children in a developing country.

BIBLIOGRAPHY

Bibliography

1. Mir TA, Ashraf M, Ahmed K, Chowdhary J, Rehana B, Ahmed J. Clinical profile, diagnostic delay, and genetic make-up of cystic fibrosis in Kashmir, India. Lung India Off Organ Indian Chest Soc. 2011;28(2):97–100.

2. Di Sant'agnese PA. Recent observations on pathogenesis of cystic fibrosis of the pancreas. Pediatrics. 1959 Aug;24(2):313–21.

3. Di Sant'agnese PA, Darling RC, Perera GA, Shea E. Abnormal electrolyte composition of sweat in cystic fibrosis of the pancreas; clinical significance and relationship to the disease. Pediatrics. 1953 Nov;12(5):549–63.

4. Shampo MA, Kyle RA. Stamp vignette on medical science. Francis S. Collins--Human Genome Project. Mayo Clin Proc. 2010 Sep;85(9):e66–7.

5. Warwick WJ, Pogue RE, Gerber HU, Nesbitt CJ. Survival patterns in cyctic fibrosis. J Chronic Dis. 1975 Dec;28(11-12):609–22.

6. Valerius NH, Koch C, Høiby N. Prevention of chronic Pseudomonas aeruginosa colonisation in cystic fibrosis by early treatment. Lancet. 1991 Sep 21;338(8769):725–6.

7. Quinton PM. Chloride impermeability in cystic fibrosis. Nature. 1983 Feb 3;301(5899):421–2.

8. Rommens JM, Iannuzzi MC, Kerem B, Drumm ML, Melmer G, Dean M, et al. Identification of the cystic fibrosis gene: chromosome walking and jumping. Science. 1989 Sep 8;245(4922):1059–65.

9. Fuchs HJ, Borowitz DS, Christiansen DH, Morris EM, Nash ML, Ramsey BW, et al. Effect of aerosolized recombinant human DNase on exacerbations of respiratory symptoms and on pulmonary function in patients with cystic fibrosis. The Pulmozyme Study Group. N Engl J Med. 1994 Sep 8;331(10):637–42.

10. Yu H, Burton B, Huang C-J, Worley J, Cao D, Johnson JP, et al. Ivacaftor potentiation of multiple CFTR channels with gating mutations. J Cyst Fibros Off J Eur Cyst Fibros Soc. 2012 May;11(3):237–45.

11. O'Sullivan BP, Freedman SD. Cystic fibrosis. Lancet. 2009 May 30;373(9678):1891–904.

12. Shastri SS, Kabra M, Kabra SK, Pandey RM, Menon PSN. Characterisation of mutations and genotype-phenotype correlation in cystic fibrosis: experience from India. J Cyst Fibros Off J Eur Cyst Fibros Soc. 2008 Mar;7(2):110–5.

13. Mei-Zahav M, Durie P, Zielenski J, Solomon M, Tullis E, Tsui L-C, et al. The prevalence and clinical characteristics of cystic fibrosis in South Asian Canadian immigrants. Arch Dis Child. 2005 Jul;90(7):675–9.

14. Bhakoo ON, Kumar R, Walia BN. Mucoviscidosis of the lung. Report of a case. Indian J Pediatr. 1968 Apr;35(243):183–5.

15. FitzSimmons SC. The changing epidemiology of cystic fibrosis. J Pediatr. 1993 Jan;122(1):1–9.

16. Kabra SK, Kabra M, Lodha R, Shastri S, Ghosh M, Pandey RM, et al. Clinical profile and frequency of delta f508 mutation in Indian children with cystic fibrosis. Indian Pediatr. 2003 Jul;40(7):612–9.

17. Nelson Textbook of Pediatrics: Expert Consult Premium Edition - Enhanced Online Features and Print, 19e: 9781437707557: Medicine & Health Science Books @ Amazon.com [Internet]. [cited 2014 Aug 3]. Available from: http://www.amazon.com/Nelson-Textbook-Pediatrics-Enhanced-Features/dp/1437707556

18. Kabra M, Kabra SK, Ghosh M, Khanna A, Arora S, Menon PS, et al. Is the spectrum of mutations in Indian patients with cystic fibrosis different? Am J Med Genet. 2000 Jul 17;93(2):161–3.

19. Hodson M, Bush A, Geddes D. Cystic Fibrosis, Third Edition. 3 edition. London: CRC Press; 2007. 486 p.

20. Blackman SM, Deering-Brose R, McWilliams R, Naughton K, Coleman B, Lai T, et al. Relative contribution of genetic and nongenetic modifiers to intestinal obstruction in cystic fibrosis. Gastroenterology. 2006 Oct;131(4):1030–9.

21. Efrati O, Nir J, Fraser D, Cohen-Cymberknoh M, Shoseyov D, Vilozni D, et al. Meconium ileus in patients with cystic fibrosis is not a risk factor for clinical deterioration and survival: the Israeli Multicenter Study. J Pediatr Gastroenterol Nutr. 2010 Feb;50(2):173–8.

22. Lykavieris P, Bernard O, Hadchouel M. Neonatal cholestasis as the presenting feature in cystic fibrosis. Arch Dis Child. 1996 Jul;75(1):67–70.

23. Moon HR, Ko TS, Ko YY, Choi JH, Kim YC. Cystic fibrosis--a case presented with recurrent bronchiolitis in infancy in a Korean male infant. J Korean Med Sci. 1988 Dec;3(4):157–62.

24. Agarwal G, Kapil A, Kabra SK, Das BK, Dwivedi SN. Characterization of Pseudomonas aeruginosa isolated from chronically infected children with cystic fibrosis in India. BMC Microbiol. 2005;5:43.

25. Molyneux ID, Morice AH. Airway reflux, cough and respiratory disease. Ther Adv Chronic Dis. 2011 Jul;2(4):237–48.

26. Heine RG, Button BM, Olinsky A, Phelan PD, Catto-Smith AG. Gastro-oesophageal reflux in infants under 6 months with cystic fibrosis. Arch Dis Child. 1998 Jan;78(1):44–8.

27. Aris RM, Merkel PA, Bachrach LK, Borowitz DS, Boyle MP, Elkin SL, et al. Guide to bone health and disease in cystic fibrosis. J Clin Endocrinol Metab. 2005 Mar;90(3):1888–96.

28. Rana M, Wong-See D, Katz T, Gaskin K, Whitehead B, Jaffe A, et al. Fat-soluble vitamin deficiency in children and adolescents with cystic fibrosis. J Clin Pathol. 2014 Jul;67(7):605–8.

29. Rosenstein BJ, Cutting GR. The diagnosis of cystic fibrosis: a consensus statement. Cystic Fibrosis Foundation Consensus Panel. J Pediatr. 1998 Apr;132(4):589–95.

30. Wescor Inc. An Elitech Company [Internet]. [cited 2014 Oct 9]. Available from: http://www.wescor.com/biomedical/cysticfibrosis/macroduct.html

31. Ashavaid TF, Raghavan R, Dhairyawan P, Bhawalkar S. Cystic fibrosis in India: a systematic review. J Assoc Physicians India. 2012 Aug;60:39–41.

32. Castellani C, Gomez Lira M, Frulloni L, Delmarco A, Marzari M, Bonizzato A, et al. Analysis of the entire coding region of the cystic fibrosis transmembrane regulator gene in idiopathic pancreatitis. Hum Mutat. 2001 Aug;18(2):166.

33. Rock MJ, Makholm L, Eickhoff J. A new method of sweat testing: the CF Quantum®sweat test. J Cyst Fibros Off J Eur Cyst Fibros Soc. 2014 Sep;13(5):520–7.

34. Alton EW, Currie D, Logan-Sinclair R, Warner JO, Hodson ME, Geddes DM. Nasal potential difference: a clinical diagnostic test for cystic fibrosis. Eur Respir J. 1990 Sep 1;3(8):922–6.

35. Moskowitz SM, Chmiel JF, Sternen DL, Cheng E, Cutting GR. CFTR-Related Disorders. In: Pagon RA, Adam MP, Ardinger HH, Bird TD, Dolan CR, Fong C-T, et al., editors. GeneReviews(®) [Internet]. Seattle (WA): University of Washington, Seattle; 1993 [cited 2014 Sep 25]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK1250/

36. Groman JD, Meyer ME, Wilmott RW, Zeitlin PL, Cutting GR. Variant cystic fibrosis phenotypes in the absence of CFTR mutations. N Engl J Med. 2002 Aug 8;347(6):401–7.

37. Strom CM, Huang D, Chen C, Buller A, Peng M, Quan F, et al. Extensive sequencing of the cystic fibrosis transmembrane regulator gene: assay validation and unexpected benefits of developing a comprehensive test. Genet Med Off J Am Coll Med Genet. 2003 Feb;5(1):9–14.

38. Wagener JS, Sontag MK, Sagel SD, Accurso FJ. Update on newborn screening for cystic fibrosis. Curr Opin Pulm Med. 2004 Nov;10(6):500–4.

39. Wells J, Rosenberg M, Hoffman G, Anstead M, Farrell PM. A decision-tree approach to cost comparison of newborn screening strategies for cystic fibrosis. Pediatrics. 2012 Feb;129(2):e339–47.

40. Farrell PM, Kosorok MR, Rock MJ, Laxova A, Zeng L, Lai HC, et al. Early diagnosis of cystic fibrosis through neonatal screening prevents severe malnutrition and improves long-term growth. Wisconsin Cystic Fibrosis Neonatal Screening Study Group. Pediatrics. 2001 Jan;107(1):1–13.

41. Rosenstein BJ. What is a cystic fibrosis diagnosis? Clin Chest Med. 1998 Sep;19(3):423–41, v.

42. Keller J, Layer P. Human pancreatic exocrine response to nutrients in health and disease. Gut. 2005 Jul;54 Suppl 6:vi1–28.

43. Lindkvist B. Diagnosis and treatment of pancreatic exocrine insufficiency. World J Gastroenterol WJG. 2013 Nov 14;19(42):7258–66.

44. Kopelman H, Durie P, Gaskin K, Weizman Z, Forstner G. Pancreatic fluid secretion and protein hyperconcentration in cystic fibrosis. N Engl J Med. 1985 Feb 7;312(6):329–34.

45. Tardelli ACS, Camargos PAM, Penna FJ, Sarkis PFB, Guimarães EV. Comparison of diagnostic methods for pancreatic insufficiency in infants with cystic fibrosis. J Pediatr Gastroenterol Nutr. 2013 Feb;56(2):178–81.

46. Walkowiak J, Herzig KH, Witt M, Pogorzelski A, Piotrowski R, Barra E, et al. Analysis of exocrine pancreatic function in cystic fibrosis: one mild CFTR mutation does not exclude pancreatic insufficiency. Eur J Clin Invest. 2001 Sep;31(9):796–801.

47. Kristidis P, Bozon D, Corey M, Markiewicz D, Rommens J, Tsui LC, et al. Genetic determination of exocrine pancreatic function in cystic fibrosis. Am J Hum Genet. 1992 Jun;50(6):1178–84.

48. Shwachman H, Lebenthal E, Khaw KT. Recurrent acute pancreatitis in patients with cystic fibrosis with normal pancreatic enzymes. Pediatrics. 1975 Jan;55(1):86–95.

49. Boeck KD, Weren M, Proesmans M, Kerem E. Pancreatitis Among Patients With Cystic Fibrosis: Correlation With Pancreatic Status and Genotype. Pediatrics. 2005 Apr 1;115(4):e463–9.

50. Sultan M, Werlin S, Venkatasubramani N. Genetic prevalence and characteristics in children with recurrent pancreatitis. J Pediatr Gastroenterol Nutr. 2012 May;54(5):645–50.

51. Midha S, Khajuria R, Shastri S, Kabra M, Garg PK. Idiopathic chronic pancreatitis in India: phenotypic characterisation and strong genetic susceptibility due to SPINK1 and CFTR gene mutations. Gut. 2010 Jun;59(6):800–7.

52. Marotta F, Labadarios D, Frazer L, Girdwood A, Marks IN. Fat-soluble vitamin concentration in chronic alcohol-induced pancreatitis. Relationship with steatorrhea. Dig Dis Sci. 1994 May;39(5):993–8.

53. Sikkens ECM, Cahen DL, Koch AD, Braat H, Poley J-W, Kuipers EJ, et al. The prevalence of fat-soluble vitamin deficiencies and a decreased bone mass in patients with chronic pancreatitis. Pancreatol Off J Int Assoc Pancreatol IAP Al. 2013 Jun;13(3):238–42.

54. Lindkvist B, Domínguez-Muñoz JE, Luaces-Regueira M, Castiñeiras-Alvariño M, Nieto-Garcia L, Iglesias-Garcia J. Serum nutritional markers for prediction of pancreatic exocrine insufficiency in chronic pancreatitis. Pancreatol Off J Int Assoc Pancreatol IAP Al. 2012 Aug;12(4):305–10.

55. Catalano MF, Sahai A, Levy M, Romagnuolo J, Wiersema M, Brugge W, et al. EUS-based criteria for the diagnosis of chronic pancreatitis: the Rosemont classification. Gastrointest Endosc. 2009 Jun;69(7):1251–61.

56. Domínguez-Muñoz JE, Alvarez-Castro A, Lariño-Noia J, Nieto L, Iglesias-García J. Endoscopic ultrasonography of the pancreas as an indirect method to predict pancreatic exocrine insufficiency in patients with chronic pancreatitis. Pancreas. 2012 Jul;41(5):724–8.

57. Zemel BS, Jawad AF, FitzSimmons S, Stallings VA. Longitudinal relationship among growth, nutritional status, and pulmonary function in children with cystic fibrosis: analysis of the Cystic Fibrosis Foundation National CF Patient Registry. J Pediatr. 2000 Sep;137(3):374–80.

58. Groleau V, Schall JI, Dougherty KA, Latham NE, Maqbool A, Mascarenhas MR, et al. Effect of a dietary intervention on growth and energy expenditure in children with cystic fibrosis. J Cyst Fibros Off J Eur Cyst Fibros Soc. 2014 Sep;13(5):572–8.

59. Corey M, Edwards L, Levison H, Knowles M. Longitudinal analysis of pulmonary function decline in patients with cystic fibrosis. J Pediatr. 1997 Dec;131(6):809–14.

60. Schaedel C, de Monestrol I, Hjelte L, Johannesson M, Kornfält R, Lindblad A, et al. Predictors of deterioration of lung function in cystic fibrosis. Pediatr Pulmonol. 2002 Jun;33(6):483–91.

61. Kerem E, Corey M, Kerem B, Durie P, Tsui LC, Levison H. Clinical and genetic comparisons of patients with cystic fibrosis, with or without meconium ileus. J Pediatr. 1989 May;114(5):767–73.

62. Sinaasappel M, Stern M, Littlewood J, Wolfe S, Steinkamp G, Heijerman HGM, et al. Nutrition in patients with cystic fibrosis: a European Consensus. J Cyst Fibros. 2002 Jun;1(2):51–75.

63. Borowitz D, Baker RD, Stallings V. Consensus report on nutrition for pediatric patients with cystic fibrosis. J Pediatr Gastroenterol Nutr. 2002 Sep;35(3):246–59.

64. Leus J, Van Biervliet S, Robberecht E. Detection and follow up of exocrine pancreatic insufficiency in cystic fibrosis: a review. Eur J Pediatr. 2000 Aug;159(8):563–8.

65. Green MR, Austin S, McClean P, Jolliffe S, Weaver LT. Spot urine pancreolauryl test for use in infancy. Arch Dis Child. 1995 Mar;72(3):233–4.

66. Kalivianakis M, Verkade HJ, Stellaard F, van der Were M, Elzinga H, Vonk RJ. The 13C-mixed triglyceride breath test in healthy adults: determinants of the 13CO2 response. Eur J Clin Invest. 1997 May;27(5):434–42.

67. Kelleher J, Walker BE, Losowsky MS. An assessment of the Van de Kamer method for estimation of faecal fat. Clin Chim Acta Int J Clin Chem. 1970 Nov;30(2):267–71.

68. Van De Kamer JH, Ten Bokkel Huinink H, Weyers HA. Rapid method for the determination of fat in feces. J Biol Chem. 1949 Jan;177(1):347–55.

69. Walkowiak J, Cichy WK, Herzig KH. Comparison of fecal elastase-1 determination with the secretin-cholecystokinin test in patients with cystic fibrosis. Scand J Gastroenterol. 1999 Feb;34(2):202–7.

70. Meyts I, Wuyts W, Proesmans M, De Boeck K. Variability of fecal pancreatic elastase measurements in cystic fibrosis patients. J Cyst Fibros Off J Eur Cyst Fibros Soc. 2002 Dec;1(4):265–8.

71. Walkowiak J. Faecal elastase-1: clinical value in the assessment of exocrine pancreatic function in children. Eur J Pediatr. 2000 Nov;159(11):869–70.

72. Toouli J, Biankin AV, Oliver MR, Pearce CB, Wilson JS, Wray NH, et al. Management of pancreatic exocrine insufficiency: Australasian Pancreatic Club recommendations. Med J Aust. 2010 Oct 18;193(8):461–7.

73. Fieker A, Philpott J, Armand M. Enzyme replacement therapy for pancreatic insufficiency: present and future. Clin Exp Gastroenterol. 2011;4:55–73.

74. Stapleton FB, Kennedy J, Nousia-Arvanitakis S, Linshaw MA. Hyperuricosuria due to high-dose pancreatic extract therapy in cystic fibrosis. N Engl J Med. 1976 Jul 29;295(5):246–8.

75. Santos CI da S, Ribeiro JD, Ribeiro AF, Hessel G. Critical analysis of scoring systems used in the assessment of Cystic Fibrosis severity: state of the art. J Bras Pneumol. 2004 Jun;30(3):286–98.

76. Shwachman H, Kulczycki LL. Long-term study of one hundred five patients with cystic fibrosis; studies made over a five- to fourteen-year period. AMA J Dis Child. 1958 Jul;96(1):6–15.

77. Taussig LM, Kattwinkel J, Friedewald WT, Di Sant'Agnese PA. A new prognostic score and clinical evaluation system for cystic fibrosis. J Pediatr. 1973 Mar;82(3):380–90.

78. Powers SW, Jones JS, Ferguson KS, Piazza-Waggoner C, Daines C, Acton JD. Randomized clinical trial of behavioral and nutrition treatment to improve energy intake and growth in toddlers and preschoolers with cystic fibrosis. Pediatrics. 2005 Dec;116(6):1442–50.

79. Kanga J, Kuhn R, Craigmyle L, Haverstock D, Church D. Cystic fibrosis clinical score: a new scoring system to evaluate acute pulmonary exacerbation. Clin Ther. 1999 Aug;21(8):1343–56.

80. ASPECTS OF THE LUNG FUNCTION DETERIORATION IN A GROUP OF PATIENTS DIAGNOSED WITH CYSTIC FIBROSIS - Google Search [Internet]. [cited 2014 Oct 5]. Available from: https://www.google.co.in/?gfe_rd=cr&ei=ELswVPjQL6PV8geEzIHoBw&gws_rd=ssl#q=ASPECTS+OF+THE+LUNG+FUNCTION+DETERIORATION+IN+A+GROUP+OF+PATIENTS+DIAGNOSED+WITH+CYSTIC+FIBROSIS

81. J. M, Y. C. Pancreatitis in Cystic Fibrosis and CFTR-Related Disorder. In: Rodrigo L, editor. Acute Pancreatitis [Internet]. InTech; 2012 [cited 2014 Oct 5]. Available from: http://www.intechopen.com/books/acute-pancreatitis/pancreatitis-in-cystic-fibrosis-and-cftr-related-disorder

82. Nousia-Arvanitakis S. Cystic fibrosis and the pancreas: recent scientific advances. J Clin Gastroenterol. 1999 Sep;29(2):138–42.

83. Davis PB, Hubbard VS, Di Sant'Agnese PA. Low sweat electrolytes in a patient with cystic fibrosis. Am J Med. 1980 Oct;69(4):643–6.

ANNEXURE

ANNEXURE - 1

IRB APPROVAL

OFFICE OF RESEARCH
INSTITUTIONAL REVIEW BOARD (IRB)
CHRISTIAN MEDICAL COLLEGE, VELLORE, INDIA.

Dr. B.J. Prashantham, M.A., M.A., Dr. Min (Clinical)
Director, Christian Counseling Center,
Chairperson, Ethics Committee.

Dr. Alfred Job Daniel, D Ortho, MS Ortho, DNB Ortho
Chairperson, Research Committee & Principal

Dr. Nihal Thomas,
MD., MNAMS., DNB (Endo), FRACP (Endo), FRCP (Edin), FRCP (Glas
Deputy Chairperson
Secretary, Ethics Committee, IRB
Additional Vice Principal (Research)

November 05, 2013

Dr. Archana Mitra. M
PG Registrar
Department of Child Health unit III
Christian Medical College
Vellore 632 002

Sub: **Fluid Research grant project:**
Pancreatic insufficiency in Indian children with Cystic Fibrosis.
Dr. Archana Mitra. M, PG Registrar, Child health unit III, Dr. Sneha Varkki, Paediatrics III, Dr. Arul Lionnel, Paediatrics, Dr. Jeyaseelan, Biostatistics.

Ref: IRB Min. No. 8492 [OBSERVE] dated 09.10.2013

Dear Dr. Archana Mitra. M,

The Institutional Review Board (Blue, Research and Ethics Committee) of the Christian Medical College, Vellore, reviewed and discussed your project entitled "Pancreatic insufficiency in Indian children with Cystic Fibrosis." on October 9, 2013.

The Committees reviewed the following documents:

1. IRB application form
2. Curriculum Vitae' of Drs. Archana Mitra. M, Sneha Varkki, Arul Lionnel, Jeyaseelan.
3. Patient consent form (English, Tamil & Hindi)
4. No of documents 1-3

The following Institutional Review Board (Blue, Research & Ethics Committee) members were present at the meeting held on October 9, 2013 in the CREST/SACN Conference Room, Christian Medical College, Bagayam, Vellore 632002.

Name	Qualification	Designation	Other Affiliations
Dr. Paul Ravindran	PhD, Dip RP, FCCPM	Professor, Radiotherapy, CMCH.	Internal, Clinician
Dr. Susanne Abraham	MBBS, MD	Professor, Dermatology, Venerlogy & Leprosy, CMCH.	Internal, Clinician
Dr. T. Balamugesh	MBBS, MD(Int Med), DM, FCCP (USA)	Professor, Pulmonary Medicine, CMCH.	Internal, Clinician
Dr. Simon Rajaratnam	MBBS, MD, DNB (Endo), MNAMS (Endo), PhD (Endo), FRACP	Professor, Endocrinology, CMCH.	Internal, Clinician
Dr. Anand Zachariah	MBBS, PhD	Professor, Medicine, CMCH.	Internal, Clinician
Dr. Ranjith K Moorthy	MBBS M Ch	Professor, Neurological Sciences, CMCH.	Internal, Clinician
Dr. Chandra Singh	MS, MCH, DMB	Professor, Urology, CMCH.	Internal, Clinician
Dr. Visalakshi	MPH, PhD	Lecturer, Dept. of Biostatistics, CMC.	Internal, Statistician
Dr. Denise H. Fleming	B. Sc (Hons), PhD	Honorary Professor, Clinical Pharmacology, CMCH.	Internal, Scientist & Pharmacologist
Dr. B. J. Prashantham	MA(Counseling Psychology), MA(Theology), Dr. Min(Clinical Counselling)	Chairperson, Ethics Committee, IRB. Director, Christian Counseling Centre, Vellore	External, Social Scientist
Dr. Ebenezer Ellen Benjamin	M.Sc, PhD	Professor, Maternity Nursing, CMCH.	Internal, Nurse
Dr. Vathsala Sadan	M.Sc, PhD	Professor, Community Health Nursing, CMCH.	Internal, Nurse

Rev. Joseph Devaraj	B. Sc, BD	Chaplaincy Department, CMCH.	Internal, Social Scientist
Mr. C. Sampath	B. Sc, BL	Advocate, Vellore, CMC	External, Legal Expert
Mrs. Pattabiraman	B. Sc, DSSA	Social Worker, Vellore	External, Lay person
Dr. Nihal Thomas	MD MNAMS DNB (Endo) FRACP(Endo) FRCP(Edin), FRCP (Glasg)	Secretary IRB (EC)& Dy. Chairperson (IRB), Professor of Endocrinology & Addl. Vice Principal (Research), CMC.	Internal, Clinician

We approve the project to be conducted as presented.

The Institutional Ethics Committee expects to be informed about the progress of the project, any **adverse events** occurring in the course of the project, any **amendments in the protocol and the patient information / informed consent**. On completion of the study you are expected to submit a copy of the **final report**. Respective forms can be downloaded from the following link: http://172.16.11.136/Research/IRB Polices.html in the CMC Intranet and in the CMC website link address: http://www.cmch-vellore.edu/static/research/Index.html.

Fluid Grant Allocation:

A sum of 60,000 INR (Rupees Sixty Thousand only) will be granted for 1 year 2 months.

Yours sincerely,

Dr. Nihal Thomas
Secretary (Ethics Committee)
Institutional Review Board

Dr. NIHAL THOMAS
MD.,MNAMS.,DNB(Endo),FRACP(Endo),FRCP(Edin),FRCP(Glasg)
SECRETARY - (ETHICS COMMITTEE)
Institutional Review Board,
Christian Medical College, Vellore - 632 002.

CC: Dr. Sneha Varkki, Child Health III, CMC.

ANNEXURE - 2

INFORMED CONSENT

Study to estimate the proportion of Pancreatic insufficiency in children with cystic fibrosis and develop clinical scoring to predict pancreatic insufficiency in CF children

Information sheet

You are being requested to participate in a study to find out the proportion of children with pancreatic insufficiency among those diagnosed to have cystic fibrosis using a special stool test –faecal elastase1. We hope to include about 60 children from this hospital in this study**.**

Why are we doing the study?

Cystic fibrosis (CF) is a genetic disorder which affects multiple organs of affected patients. It causes obstruction and infection of airways and indigestion. CF though rare in India, affects many children and causes severe chronic lung disease and pancreatic insufficiency. About 85% of children with cystic fibrosis are found to have pancreatic insufficiency in western studies. In our country tests to identify pancreatic insufficiency are not regularly done as they are costly and available only in few places. It is proven that early identification of pancreatic insufficiency and appropriate treatment for the same

would improve the lung function and quality of life in children with cystic fibrosis. In future we may be able to detect the children with pancreatic insufficiency based on major clinical symptoms and start early treatment.

What is your role in the study?

If you agree to participate in this study, your child will be submitted to a questionnaire which includes medical history and examination with specific regard to pancreatic insufficiency. We will test stool for fecal elastase which is low in children with pancreatic insufficiency .We will also include blood tests and simple stool test that provides us an additional clue for pancreatic insufficiency. No special preparation is necessary for the above tests.

Will you have to pay for the test?

Tests which are done for routine care of the patient will have to be paid. However stool elastase test can be done at a subsidised rate for study patients.

Can you withdraw from this study after it starts?

Your participation in this study is entirely voluntary and you are also free to decide to withdraw permission to participate in this study. If you do so, this will not affect your usual treatment at this hospital in any way.

What do I benefit from the study?

You may benefit from the results of the study. If we identify that your child has pancreatic insufficiency he/she can be started on pancreatic supplements which will

improve your childs nutritional status. This in addition has been found to improve your childs lung status .

The scoring system we develop from this study will help to identify patients with pancreatic insufficiency even when costly tests cannot be done.

.Will your personal details be kept confidential?

The results of this study may be published in a medical journal or shared in a scientific meeting. However you will not be identified by name in any publication or presentation of results. Your medical notes may be reviewed by people associated with the study, without your additional permission, should you decide to participate in this study.

If you have any further questions, please ask

Dr. M. Archana Mitra (0416-2283343),

Dr. Sneha Varkki (0416-2283343)

Dr. Arul Lionel (0416 2283350)

email: child3@cmcvellore.ac.in

ANNEXURE - 3

CONSENT TO TAKE PART IN A CLINICAL TRIAL

Study Title: ***Pancreatic insufficiency in children with cystic fibrosis and clinical scoring to predict pancreatic insufficiency in children with cystic fibrosis***

Study Number:

Participant's name:

Date of Birth / Age (in years):

I_____

_____, father/ mother/ guardian of _____

(Please tick boxes)

Declare that I have read the information sheet provided to me regarding this study and have clarified any doubts that I had. [ ]

Declare that the information sheet has been read to me regarding this study and I have clarified any doubts that I had.[ ]

I also understand that my child's participation in this study is entirely voluntary and that I am free to withdraw permission to continue to participate at any time without affecting my child's usual treatment or his/her legal rights [ ]

I understand that the study staff and institutional ethics committee members will not need my permission to look at my child'shealth records even if I withdraw from the trial. I agree to this access [ ]

I understand that my child's identity will not be revealed in any information released to third parties or published [ ]

I voluntarily agree for my child to take part in this study [ ].I hereby give permission for stool examination of my child for fecal elastase.

Name:

Signature:

Date: **Area for thumb impression**

Name of witness:

Relation to participant:

Date:

ANNEXURE - 4

CHILD'S ASSENT TO TAKE PART IN A CLINICAL TRIAL

Study Title: Pancreatic insufficiency in children with cystic fibrosis and clinical scoring to predict pancreatic insufficiency in children with cystic fibrosis

Study Number:

Participant's name:

Date of Birth / Age (in years):

I_____

_____, son/daughter of _____

(Please tick boxes)

Declare that I have read/read to me the information sheet provide to me regarding this study and have clarified any doubts that I had. [ ]

I also understand my participation in this study is entirely voluntary and that I am free to withdraw permission to continue to participate at any time without affecting my child's usual treatment or his/her legal rights [ ]

I understand that the study staff and institutional ethics committee members will not need my permission to look at my health records even if I withdraw from the trial. I agree to this access [ ]

I understand that my identity will not be revealed in any information released to third parties or published [ ]

I voluntarily agree to take part in this study [ ].I hereby give permission for my stool examination for fecal elastase.

Name:

Signature:

Date:

ANNEXURE – 5

TAMIL CONSENT

அனுமதி படிவம்

கிறித்துவ மருத்துவ கல்லூரி, வேலூர்
குழந்தைநல பிரிவு

தகவல் தாள்

சிஸ்டிக் பைப்ரோஸிஸ் என்ற கணய நோய் பாதிப்பின் சம்பந்தத்தை அறியும் ஆய்வு.

சிஸ்டிக் பைப்ரோஸிஸ் என்ற வியாதியில் கணய பாதிப்பின் சம்பந்தத்தை அறியும் ஆய்வில் உங்களை பங்கு கொள்ள அழைக்கிறோம். இந்த பாதிப்பு பீக்கல் எலாஸ்டேஸ் (faecal elastase) என்ற அறிய பரிசோதனை மூலம் கண்டுபிடிக்கப்படும். இந்த மருத்துவமனையில் 60% குழந்தைகள் பங்கு பெறுவார்கள் என எதிர்பார்க்கிறோம்.

இந்த ஆய்வின் நோக்கம்

சிஸ்டிக் பைப்ரோஸிஸ் (சி.எப்.) நோயினால் பாதிக்கப்பட்ட நோயாளிகளின் பல உறுப்புகளை பாதிக்கும் ஒரு மரபணு குறைபாடு ஆகும். இந்தியாவில் சி.எப். அரிய நோயாக இருந்தாலும் பல குழந்தைகள் (சி.எப்.) நோயினால் மூச்சுக்குழாயில் அடைப்பு மற்றும் அஜீரணத்தினால் பாதிக்கப்பட்டிருக்கிறார்கள். சிஸ்டிக் பைப்ரோஸிஸ் நோயால் பாதிக்கப்பட்ட 85% குழந்தைகள் கணய பாதிப்பினால் அவதிப்படுவதை காணலாம். பரிசோதனைகளுக்கு நிறைய செலவாவதாலும் நம் நாட்டில் வெகு குறைவான இடங்களிலே தவிர பொதுவாக இதற்கான பரிசோதனைகள் செய்யப்படுவதில்லை. சிஸ்டிக் பைப்ரோஸிஸ் நோயினால் பாதிக்கப்பட்ட குழந்தைகளின் கணய பாதிப்பு இருப்பது சீக்கிரமாக அறிந்து கொள்வது மிகவும் நல்லது. ஏனென்றால் விரைவான சிகிச்சை அளிப்பதன் மூலம் அந்த குழந்தையின் வாழ்க்கை தரமாக இருக்கும்.

ஆய்வில் உங்கள் பங்கு என்ன?

இந்த ஆய்வில் பங்கு பெற நீங்கள் சம்மதித்தால் நாங்கள் உங்களுடைய குழந்தையின் மருத்துவ விவரங்களை எடுத்து உங்கள் குழந்தையை ஆய்வு செய்வோம். இந்த ஆய்வில் தங்கள் குழந்தையின் ஃபீக்கல் எலாஸ்டேஸ் (faecal elastase) பரிசோதனை செய்து கணய பாதிப்பு உள்ளதா என்று பார்க்கப்படும். கூடுதலாக சாதாரண பிற இரத்த பரிசோதனைகள் மற்றும் மலம் பரிசோதனை செய்வோம். இதற்கு சிறப்பு தயாரிப்பு எதுவும் தேவையில்லை.

நீங்கள் இந்த ஆய்வுக்கு பணம் செலுத்தவேண்டுமா?

ஃபீக்கல் எலாஸ்டேஸ் (faecal elastase) பரிசோதனைக்கு குறைந்த கட்டணம் வசூலிக்கப்படும். இதை தவிர மற்ற சாதாரண பரிசோதனைகளுக்கு வழக்கம் போல பணம் வசூலிக்கப்படும்.

ஆய்வு தொடங்கிய பிறகு இதிலிருந்து விலகிக்கொள்ள முடியுமா?

இந்த ஆய்வில் உங்கள் பங்கு முற்றிலும் தன்னிச்சையானது. நீங்கள் விருப்பப்பட்டால் இதிலிருந்து எப்போது வேண்டுமானாலும் விலகிக் கொள்ளலாம். இதனால் இந்த மருத்துவமனையில் உங்கள் வழக்கமான சிகிச்சை எவ்விதத்திலும் பாதிக்காது.

இந்த ஆய்வினால் ஏற்படும் நன்மை:

பரிசோதனையில் குணய பாதிப்பு ஏதேனும் இருப்பின் அதை இந்த ஆய்வில் கண்டுபிடிக்கமுடியும். இதனால் தங்களின் குழந்தைக்கு தகுந்த சிகிச்சை அளிக்கப்படும். அதன் மூலம் தங்கள் குழந்தையின் நுரையீரலின் தரமும் பாதுகாக்கப்படும்.

உங்களின் தனிப்பட்ட விவரங்கள் பாதுகாக்கப்படுமா?

இந்த ஆய்வின் முடிவுகளை ஒரு மருத்துவ நாளேட்டில் அல்லது விஞ்ஞான கூட்டத்தில் பகிர்ந்து கொள்ளப்படலாம். உங்களின் கூடுதல் அனுமதியின்றி உங்கள் விவரங்களையும் ஆய்வின் முடிவுகளையும் தொடர்புடைய நிபுணர்களோடு கலந்தாயப்படும்.

உங்களுக்கு இது தொடர்பாக சந்தேகம் இருந்தால் நீங்கள் தொடர்பு கொள்ள வேண்டிய முகவரி:-

டாக்டர். அர்ச்சனா மித்ரா
டாக்டர். ஸ்நேகா வர்க்கி
டாக்டர். அருள் பிரேமானந்த் லயனில்

தொலைபேசி: 0416 2283343
மின்னஞ்சல்: child3@cmcvellore.ac.in

ஒப்புதல் படிவம்

கணைய நோய் பாதிப்புள்ள குழந்தைகளின் சிஸ்டிக் பைப்ரோஸிஸ் மற்றும் கிளினிகல் ஸ்கோரிங் சம்பந்தத்தை அறியும் ஆய்வு.

ஆய்வு எண்
குழந்தையின் பெயர்
பிறந்த தேதி/வயது (வருடங்கள்)

நான் ..... மகன்/மகள் .....

(தயவு செய்து கட்டத்தில் [√] குறியிடவும்)

எனக்கு தரப்பட்ட தகவல் தாளை முழுவதுமாக படித்து சந்தேகங்களை தெளிவுபடுத்திக் கொண்டேன் [ ]

எனக்கு தரப்பட்ட தகவல் தாளில் உள்ள விவரம் படித்துக் காட்டப்பட்டது. அதிலுள்ள சந்தேகங்களும் தெளிவுபடுத்தப்பட்டது.
[ ]

இந்த ஆய்வில் என் குழந்தையின் பங்கேற்பு தன்னிச்சையானது என்றும் எந்த நேரத்திலும் இதிலிருந்து விலகிக்கொள்ளலாம் அதனால் என்னுடைய குழந்தையின் வழக்கமான சிகிச்சை பெறுதல் எந்த வகையிலும் பாதிக்கப்படாது என்பதையும் புரிந்துகொண்டேன்.
[ ]

இந்த குழுவினை சார்ந்த பணியாளர்களோ மற்றும் மருத்துவ குழு உறுப்பினர்களோ நான் இந்த ஆய்விலிருந்து விலகிக்கொண்டாலும் என் குழந்தையின் மருத்துவ படிவங்களை பார்வையிட என்னுடைய அனுமதி பெற தேவையில்லை என்பதை புரிந்துகொண்டேன்.
இதற்கு நான் சம்மதிக்கிறேன். [ ]

என்னுடைய குழந்தையை பற்றிய விவரங்களை வெளி நபர்களிடம் தெரிவிப்பதோ அல்லது மூன்றாம் நபர்களிடம் வெளியிடவோ மாட்டார்கள் என்பதை புரிந்து கொண்டேன். [ ]

இந்த ஆய்வில் என் குழந்தை பங்கு கொள்ள தன்னிச்சையாக சம்மதிக்கிறேன் [ ]. ஃபீக்கல் எலாஸ்டேஸ் (faecal elastase) பரிசோதனைக்கு உட்பட தேவையான மலத்தை என் குழந்தை தர சம்மதிக்கிறேன்.

பெயர் : **கைவிரல் ரேகை பதிவு**
கையொப்பம் :
தேதி:

ANNEXURE - 6

PROFORMA

NAME: **Hosp No.** **Date**

AGE (DOB):

SEX

ADDRESS

SYMPTOMS	**Age first noted**
Meconium ileus, intestinal obstruction	**yes / no**
Malabsorption - oily stools	**yes / no**
Failure to thrive, malnutrition	**yes / no**
Rectal prolapse	**yes / no**
Salty taste	**yes / no**
Salt craving	**yes / no**
Hepatobiliary disease	**yes / no**
Metabolic alkalosis /pseudobartter	**yes / no**

Recurrent dehydration **yes / no**

Diabetes mellitus (> 10yrs)

Others

Birth weight **current weight**

∆weight loss

Symptoms of failure to thrive **yes/no**

Gastrointestinal symptoms:

Foul smelling stools

Bulky and oily stools

Frequent stools more than twice per day **yes/no**

Abdominal distension

Recurrent abdominal pain

Nausea and vomiting

Number of hospital admissions for diarrhoea in the past

History of documented hypoglycaemia

Symptoms related to fat malabsorption

History of fractures

Treatment History: **Duration**

Pancreatic supplements **yes / no**

How long after Dx treatment started

ATT given

Vitamin supplements

Anti acid drugs

Anti reflux drugs

Family History

Family history of pancreatitis **yes / no**

Male Infertility **yes / no**

Environmental factors

Smoking **yes / no**

Examination:

<u>Growth:</u> **Centile**

Weight-

Height-

BMI

Chest circumference

Head circumference if < 2 years

Mid arm circumference

Skin fold thickness

General examination

Vitamin deficiencies:

Vitamin A **yes / no**

Vitamin D **yes / no**

Clubbing **yes / no**

Edema **yes / no**

Epigastric mass/tenderness **yes / no**

Investigations

Sweat Chloride test

Haemoglobin

Serum albumin

PT, aPTT

Chest X-ray

Serum Calcium

Vit D level

CFTR Mutation

Stool fat globules

Fecal elastase

ANNEXURE - 7

COOPERMAN SCORE

CATEGORIES	2	1	0
Activities	Normal upon physical exertion; Engages in typical physical activities	Regular school attendance (maximum, 2 absenses/month)	
Chest X-ray	Normal	Slightly increased markings; Emphysema	
Digital Clubbing	0 to 1+	1 to 2+	2+ (extensive)
Growth and Development	Height and Weight above the 25th percentile	Height and Weight above the 3rd percentile	Height and Weight below the 3rd percentile
Complications	None	Transitory	perpetual

ANNEXURE 8

METHODOLOGY OF FECAL ELASTASE

Reagents needed for test

1) Reference standard set per vial-0.7ml

--Standard 1- 50µg elastase/g

--Standard 2- 100µg elastase/g

--Standard 3- 200µg elastase/g

--Standard 4 -500µg elastase/g

2) Control 1(equivalent to 90µg elastase/g +/- 20%) containing 0.2% sodium azide- 0.7ml

3) Control 2(equivalent to 200µg elastase/g+/-20%) containing 0.2% sodium azide- 0.7ml

4) Washing solution (10X concentrated) - 2X 50ml

5) Extraction buffer (10X concentrated)- 50ml

6) Biotinylated elastase antibody- 0.12ml

7) Streptavidin peroxidase conjugate- 8ml

8) Substrate solution of TMB- 13ml

9) Stop solution of H2SO4- 13ml

10) Microstrips coated with polyclonal anti elstase antibody

Specimen Collection and storage: Fresh stool sample are collected for faecal elastase 1 testing. Samples are transported to lab immediately and stored at different temperatures for the following time spans.

Environmental temperature up to 40°C or 104°F for up to five days.

Refrigerator temperature 2°C -8°C(36°F-46°F) for upto one week.

Household freezer temperature -18°C_-20°C(0°F to -4°F) for upto one year.

Preparation of Faeces sample:

Preparation of Extraction buffer: The Extraction buffer is diluted with distilled or deionised water in 1: 10 dilution.

Weighing of Faeces samples:

A 12ml tube is used with a spatula to weigh 30-100mg stools on a scale with a sensitivity of 1mg. 1ml of extraction buffer per 10mg faeces.The samples are homogenised thoroughly with a vortex mixer for 2 minutes.After sedimentation of solid constituents, the supernatant can be used for the determination of the elastase after dilution.

Assay procedure:

1) The concentrated washing solution must be diluted with 450ml of distilled or de ionised water
2) All the reagents are warmed to room temperature and mixed thoroughly before use.
3) Required number of coated wells or strips in the strip holder are fixed.
4) The supernatants of extracted faeces are diluted in 1: 201 with washing solution.
5) 50µL of the blank elastase standards and controls are dispensed each extracted and diluted faeces samples wih new disposable tips into wells
6) These wells are incubated for 60 minutes at room temperature.
7) The contents are shaken out of the strips and the wells ar rinsed 3 times with 200µL washing buffer each.

8) The residual water is knocked out of the wells by hitting them on absorbent paper or cloth

9) The biotinylated second anti elastase antibody is diluted in 1:201 dilution with washing solution

10) This is incubated for 30min at room temperature.

11) Later the contents are shaken out of the strips and the wells are rinsed 3 times with washing solution, each time using 200µL.

12) The residual water is then knocked out of the wells by hitting them on adsorbent paper or cloth.

13) 50µL of streptavidin peroxidase conjugate is dispensed into each well.

14) This is again incubated at room temperature for 30min.

15) The contents are again shaken out of the strips and the wells are rinsed 3 times with washing solution each time using 200µL

16) The residual water is then knocked out of the wells onto the adsorbent paper or cloth

17) 100µL of substrate solution is dispensed into each well and incubated for 20minutes at room temperature

18) Now the enzymatic activity is stopped by adding 100µ of stop solution into each well.

19) The absorbence of each well is read at 450nm with a microplate reader withi 10 minutes of stopping the enzymatic reaction.

Pipetting scheme for the pancreatic Elastase ELISA in the lab

	1	2	3	4	5	6	7	8	9	10	11	12
A	**Blank**	**blank**	**P**	**2**	**P**	**10**	**P**	**18**	**P**	**26**	**P**	**34**
B	**S**	**1**	**P**	**3**	**P**	**11**	**P**	**19**	**P**	**27**	**P**	**35**
C	**S**	**2**	**P**	**4**	**P**	**12**	**P**	**20**	**P**	**28**	**P**	**36**
D	**S**	**3**	**P**	**5**	**P**	**13**	**P**	**21**	**P**	**29**	**P**	**37**
E	**S**	**4**	**P**	**6**	**P**	**14**	**P**	**22**	**P**	**30**	**P**	**38**
F	**C**	**1**	**P**	**7**	**P**	**15**	**P**	**23**	**P**	**31**	**P**	**39**
G	**C**	**2**	**P**	**8**	**P**	**16**	**P**	**24**	**P**	**32**	**P**	**40**
H	**P**	**1**	**P**	**9**	**P**	**17**	**P**	**25**	**P**	**33**	**P**	**41**

In the pipetting scheme the recommended positions for the blank (zero standard) , standards (S1-S4), Controls (C1 and C2) and for the patients samples (P1-P41) are shown as double determinations.

ABBREVATIONS

CF	Cystic Fibrosis
PI	Pancreatic insufficiency
PS	Pancreatic Sufficiency
FE	Fecal Elastase
PERT	Pancreatic enzyme replacement therapy
CFTR	Cystic Fibrosis Transmembrane Regulator
ABP	Abdominal Pain
ABN	Abdominal Distension